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Information to Contributors

Purpose and Scope

Part B of the Proceedings is devoted to papers embodying results of original research in any branch of Life Sciences. The major emphasis of the journal will be on publication of studies especially those with Inter-disciplinary implications relating to Physiology, Cell Biology, Cytogenetics, Genetics, Ecology, Biochemistry, Microbiology, Pathology, Endocrinology, Physiology of Reproduction, etc., relating to plants and animals.

Papers presented at the Academy's symposia, special Academy lectures, original review articles relating to research and concepts fundamental to Life Sciences are also published in the Proceedings.

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ANNIVERSARY ADDRESS
Genetics Today and Tomorrow

A K SHARMA
President, Indian National Science Academy, New Delhi 110 002

(Delivered 2 January 1985)

Advances in the science of genetics, the only unifying discipline in biology, have been spectacular in the last three decades. The genes have been chemically resolved, isolated, synthesized and subjected to manipulation through techniques of genetic engineering.

The scope of Genetics covers a wide spectrum, ranging from microbes to man, and the advances in each level of hierarchy have been phenomenal. In the microbial system, the prokaryotes, the gene-bearing structure is a long-chain DNA molecule named genophore. The study of gene-expression in a bacterial or a viral system involves merely the direct analysis of gene products at the level of the gene itself. The entire structure is extremely simple, being unicellular, and all activities are just confined within the small unit.

In higher organisms — plants, animals and man, the genes in the DNA molecule are organized into complex structures the chromosomes, which in addition to DNA, contain basic and non-basic proteins. The genes in chromosomes are *split* consisting of essential and non-essential sequences. Over and above, is the fact, that a heavy percentage of DNA, in the higher organism is composed of repeated sequences, the functions of which are still obscure. Such sequences as noted different centres of research including that of our own, may represent 2/3rd to 3/4th of the total amount of DNA in the organism. Their function is still an enigma in biology.

In higher organisms, the body being multicellular and highly differentiated, each and every character is the ultimate result of a series of chemical reactions initiated, originally at the gene level. As such, several intermediate steps intervene between the ultimate effect, which is analysable, and the levels of genes where the action is initially triggered. This poses tremendous complexity in the analysis of gene-expression in eukaryota as compared to that of prokaryotes.

Genetic Manipulation

Genetics has come to the forefront of scientific disciplines due to tremendous potential of the methodology developed for genetic manipulation. Leaving aside hybridization and recombination, tinkering with the gene substance owes its initiation to the discovery in 1927 by Muller demonstrating that irradiation can induce gene alterations in biological systems. Mullers' discovery led to the establishment of the science of Radiation Genetics and later, Physical and Chemical mutagenesis — no doubt operating in a non-directed fashion.

Genetic Manipulation techniques in recent years have undergone much refinements through the use of enzymes needed for cutting and joining the DNA segments respectively at specific loci. These principally involve, introduction after isolation of DNA into the recipient bacterium, cutting the specific sequences of donor DNA and self-replicating plasmid molecule through restriction enzymes, joining the donor sequences in plasmids through ligases, followed by maintenance of the plasmid in the bacteria for cloning or propagation. The same process can be repeated with the incorporated gene in the plasmid which can be further taken off from the plasmid and introduced into the genophore of the bacterium. In suitable medium, the bacterium can propagate and the genes incorporated in it either in the plasmid or ultimately in the genophore, can find expression in the synthesis of coded enzymes.

Impact in Microbial System

The impact of recombinant DNA technique varies on microbes, plants and animals. In microbes, the organisms *per se* are deeply involved in the methodology, serving not only as vectors but also as the substrate for cloning and synthesis of the products. The genetic architecture of the organism as a whole, undergoes alteration. Recombinant DNA technique coupled with the fusion of bacterial cells are extensively made use of in conferring the property of production of antibiotics from one bacterium to another or the production of hybrid antibiotics. Similarly, the capability of nitrogen-fixation of *Klebsiella* has been transferred to other bacteria which otherwise possess no such property. The hydrocarbon-degrading capacity of the microbial genes has been cloned in a single bacterium through successive cutting and joining by specific enzymes.

Besides, in view of the simplicity of the organism, its capacity to act as vector, absorb foreign gene in its architecture, and to act as a cloning and expressing medium, the microbes offer enormous possibilities for genetic manipulation, which, with further refinements, could be carried out with predictable accuracy. The impact of these researches on degradation of pollutants and purification of environment is enormous.

Impact in Animals and Man

In animals, in general, and man, in particular, there have been remarkable advances from a fundamental standpoint. A large number of human genes have been mapped and located in specific chromosomes. Elegant techniques, have been devised, including extraction and molecular hybridization leading not merely to identification of loci but their functional significance as well.

It is now possible to make a precise diagnosis of all the inherited conditions of haemoglobin through DNA analysis. Prenatal diagnosis of genetic disorders with the aid of DNA probes has emerged as a very powerful tool in Human Genetics. With the availability of more genes for cloning, it would be possible to utilize them as probes for the diagnosis of diseases with unidentified defective structural genes. The faetal sex determination is at present a simple technique utilizing identification through specific probes of "Y-chromosomes". Nearly 30 distinct types of neoplasia have been identified with 22 specific chromosomal defects. Advances in different aspects of human genetics *per se* have led to a better understanding of the fundamental genetic structure, the gene functions, and subsequently the method for the detection of disorders.

The recombinant DNA technique has given a new dimension to pharmacology to a great extent, the best example being the production of insulin in a bacterial system. The isolation of insulin-coding gene from mammals, its incorporation into

the bacterium through a plasmid vector and ultimately the production of insulin in the bacterium serving as a factory, are established facts. Similar possibilities do exist for a wide range of interferons, the antibody proteins, which are specific for a large number of diseases, including possibly cancer. The capacity of the bacterium to accept the genes of higher organisms and act as a suitable medium of their expression are the key factors in these operations. Ultimately, it amounts to utilization of the bacterium as a production centre for a variety of drugs including insulin, interferons, human hormones and a host of pharmaceuticals. Simultaneously, the fusion of lymphocytes with antibody-producing cells—the hybridoma technology—has paved the way for the production of monoclonal antibodies.

Thus, in the mammalian system, emphasis has been laid towards three different approaches; (*i*) identification of genes in chromosomes, (*ii*) diagnosis of genetic disorders, and finally (*iii*) the production of drugs through the introduction of desired gene in the bacterium. Nowhere there has been an alteration in the genetic architecture of the individual or the introduction of foreign genes as in case of prokaryotes. excepting in melignancy.

The coming decade would undoubtedly witness much more developments in this direction utilizing bacteria as a medium for production of a variety of drugs. But the ultimate objective of curing the genetic disorder at the genetic level is yet to be attained. This dimension of research which does not contemplate the production of drug in a bacterial factory in the artificial medium but visualizes its production in the human body through the incorporated gene is yet to make a solid base. It implies, the isolation of interferon or insulin-coding gene from the donor and its introduction to the human recipient, ensuring continuous production of the drug in the human system. Such incorporation may involve the functioning of the recombinant bacterium as a factory within human system or ultimately the introduction of the gene in the human chromosome itself. With the present rate of development of technology, the recombinant bacteria serving as drug production centre in human organ instead of *in vitro* may be possible in near future, though not necessarily the introduction of foreign gene in the chromosomes of man.

Impact in the Plant System

In the plant system, there has been phenomenal advances in different facets of genetics. The identification and analysis of fine structure of chromosome, study of sequence complexity, including additional genetic elements, population analysis, improvement through breeding, mutation and chromosome substitution techniques have paid very high dividends both from fundamental and utilitarian standpoints. But recombinant DNA technique in this case, is designed to achieve the objective of restructuring plants own genetic component through introduction of gene by specific enzymes as in the case of bacteria. The execution of this task in plants is fraught with innumerable limitations as compared to that in microbes where the task is quite simple as the object is unicellular with no differentiation.

One of the advantages of the plant system is its totipotency, which theoretically enables any cell to regenerate into a whole plant in the proper medium. Significant advances have been made in cell, tissue, and organ culture which have led to production of innumerable plants of desired genetic constitution without involving the complicated process of sexual reproduction and fertilization. In fact, induction of mutation followed by the regeneration in culture has become an effective tool in securing improved variety of crops within

a short-span of time which through conventional breeding practices would have taken benerations to achieve. One can secure haploid cells in culture, induce desired mutations through a random mutagenic treatment and regenerate the selected individuals in multiples. Further, regeneration of homozygous plants through artificial induction of doubling assures the purity and stability of the individual, One can hardly doubt the phenomenal advances made and the potential of *in vitro* technique for propagation and improvements in agriculture, horticulture and forestry.

However, the other facet of genetic manipulation in which foreign genes either from different plants or from microbes, need direct introduction into the recipient chromosome through recombinant DNA technology as in bacteria suffers from serious constraints. Lately, a case of successful transfer of protein 'Phaseolin' from *Phaseolus* to sunflower, has been reported.

Most of the works so far carried out deal with the insertion of genes from higher organisms into the microbial recipient where the enzymes coded by the former for pharmaceuticals, and drugs like insulin, interferon and growth hormones are expressed. There has been only one single example of the insertion of a bacterial gene for antibiotic resistance into *Petunia* cells in culture.

The failure in direct introduction of the genes of a microbe or a plant to another plant through recombinant DNA technique can be attributed to various factors. Plant genome is exceedingly complex involving at least 5 to 6 million genes in an average crop plant. A large number of genes are located outside the chromosome such as in chloroplastids and mitochondria. Each gene has three regions—the promoter, the series of nucleotides coding for the enzyme the structural sequences and the terminators. All these sequences require identification before introduction of a foreign gene in the system.

One of the crucial factors for a plant geneticist is the identification of a suitable vector for carrying the passenger or foreign gene into a suitable recipient system. The protoplast being naked (devoid of cell wall), and theoretically having the capacity of regeneration, is a convenient recipient medium. However, though potatoes of *alpha alpha* as well as several Solanaceous plants grow very well from the protoplast, others like corn, wheat or soybeans do not do so. The technique therefore calls for further improvements for universal applicability.

Of the vectors moreover, the only one demonstrated to be effective is the Ti plasmid associated with *Agrobacterium tumafaciens* —a bacterium responsible for crown gall in legumes. But its application restricted the specificity being mainly confined to species infected by Agrobacterium alone. Its range is to be further widened by genetic manipulation so that it can be utilized to carry genes to cereals from grasses or from bacteria too. Of the other vectors, only cauliflower mosaic virus appears promising.

The key to the success of genetic engineering in higher organisms is a precise understanding of the mechanism of control of differentiation. Each and every cell contains the same genetic complement but growth and differentiation being sequential and phasic, only certain genes are switched on at the proper time, the others being shut off during that period. Genes for photosynthesis are present in all organs but expressed only in the leaf and not in the root. An understanding of this mechanism is a prerequisite to the identification of the control elements regulating gene expression.

Even after a foreign gene is inserted in the living system through a vector, it is difficult to ensure that it would be expressed only in the organ needed and not elsewhere. The issue is further complicated by the fact that a character in a plant is not necessarily controlled by one or many gene(s) in a

single chromosome, but rather they are more likely to remain distributed in different chromosomes. The basis for their simultaneous functioning responsible for the harmonious expression of the character is not yet fully understood. The manifestation of the tremendous potential of plant genetic engineering would await an understanding of the basic factors involved in regulation of differentiation with the inherent biochemistry and physiology of regulating factors. Theoretically, there is potential of isolation and incorporation of the genes for resistance to herbicide or drought or capacity to thrive in marginal, salty or acidic soil, to crops and commercially important species. In relation to nitrogen fixation, where 17 genes are involved in bacteria, recombinant strains with the capacity for fixation or accelerated fixation either as free-living organisms or as symbionts in legumes are demonstrable. But the transfer of these genes with their individual control elements from the bacterium to the plant presents innumerable fundamental problems. The potential is unlimited but what is needed is a basic understanding of the genetic control of differentiation.

One can visualize advances in the methodology for the transfer of genes from one plant to another in view of the similarity in the nature of the genes — being split in both, and similarity of the control signals. The heavy amount of additional DNA offers enormous scope of utilization of these sequences in genetic manipulation techniques.

Future Scope

The coming decade would undoubtedly witness major advances in genetics vis-a-vis recombinant DNA technology. The identification of almost all human genetic disorders through random DNA probes is within the realm of possibility. More bacterial factories would be established for the production of insulin, interferons, hormones as well as other pharmaceuticals. One can look forward to a rapid method for environmental purification with the aid of more powerful recombinant organisms. Nitrogen-fixation through more efficient microbes harbouring the cereals would undoubtedly be achieved in near future.

The cell, tissue and organ culture including the protoplast has opened up new vistas for rapid propagation, enrichment of genetic diversity and introduction of genes in the system. Protoplast fusion would enable the regeneration of novel hybrids in plants, unimaginable in the history of conventional hybridization. The impact of these methodologies in the improvements of cereals, medicinal and commercially valuable species, can hardly be overrated.

But the ultimate objective of altering the genetic architecture of a plant for improvement in agriculture or the human organ for amelioration of diseases, through recombinant DNA technology, may not possibly be attained in near future. A prerequisite for the realization of this aim is a basic understanding of the control signals regulating differentiation in eukaryota. The sequential and phasic growth and diversification of organs, in the higher hierarchy of evolution, is one of the most challenging problems in biology. With the intensification of researches on genetics, biochemistry and physiology, one can hope to have in near future, a deep insight into the fundamentals of the genic and chromosomal control of differentiation in higher organisms. This will pave the way in later years for the introduction of genes in the genetic architecture of higher eukaryotes. If achieved, even though in distant future, it will be a remarkable feat of genetic engineering — fulfilling the very objectives of a scientist — search for truth, enrichment of qualily of life, and alleviation of human suffering.

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Scanning Electron Microscopic Studies of Wing Microsculpturing in Termites (Isoptera). I. Genera *Heterotermes* and *Microtermes*

M L ROONWAL

Desert Regional Station, Zoological Survey of India, Paota B Road, Jodhpur

(Received on 24 September 1984)

In earlier light microscope (LM) studies an extensive comparative survey of wing microsculpturing (with at least eight types of structures occurring) in all the major families and subfamilies of the order Isoptera was carried out (Roonwal & co-workers 1967–1983). In this series are presented the results of scanning electron microscope (SEM) studies, the first on wing microsculpturing. In the present part two genera, *Heterotermes* Froggatt (Rhinotermitidae, Heterotermitinae) and *Microtermes* Wasmann (Termitidae, Macrotermitinae), are dealt with.

Heterotermes: *H. indicola* was studied. Micrasters here consist of three main types, viz. simple rods (very rare), V-shaped, thin, flowery or leafy, sheath-like bodies with 0–3 pistil-like rods projecting out (most common, 93%) and asteroids with 5–7 arms. The asteroids seem to have evolved from the leafy V-bodies, and intermediate forms are sometimes present. Sizes about 4 to 7 μm.

Microtermes: *M. obesi* was studied. SEM images revealed that the microsculpturing on the membrane should be classed as simple, nonasteroid micrasters (not as rods as regarded earlier). They are of two main types, viz. one-armed; and a V-shaped, leafy sheath-type with sometimes a central, pistil-like arm sprouting out; V-shaped bodies predominate (77%). Their relationship with microsculpturing in other termites, especially the macrotermitine genera, is discussed.

Key Words: **Wing microsculpturing, Scanning electron microscope images, Termites, Isoptera,** *Heterotermes, Microtermes*

Introduction

In recent years, the existence of a dense carpet of various types of cuticular, microsculpturing elements on both upper and lower surfaces of termite wings has been established (Roonwal & co-workers 1967-1983, vide the references). Nearly 80 genera and over 240 species, belonging to all the major families and subfamilies, have been studied. At least eight main types of these elements (besides hairs) are now known and have been termed, according to their general shape and structure, as papillae, arrowheads, tubercles, spearheads, pimpules, micrasters, microsetae and rods. Their size varies from ca. 0.5 to 24 μm, and density from low to over 12500/mm^2, and more than half a

million of them may be present on a single wing surface. All the eight types are not present simultaneously in a species, but papillae are present universally in all. Of the remainder, not more than two may be present, or none at all. Their phylogeny, taxonomic value and biological and ecological significance have been discussed by Roonwal (1983 a, b). These structures would seem to be unique to termites.

The studies mentioned above were made with light microscopes (LM). Recently, scanning electron microscope (SEM) facilities became avaliable, and the structures were re-examined. The results are given in the present series. While in general the SEM studies confirm the LM findings, the intimate details of the individual elements so revealed throw fresh light on them, especially as regards their true shape, structure, component parts, etc.

Results

1 Genus HETEROTERMES Froggatt
(Rhinotermitidae, Heterotermitinae)

LM studies on *Heterotermes* have been published by Roonwal and Verma (1976, *H. gertrudae*, micrasters), Roonwal and Rathore (1977, *H. gertrudae*, *H. indicola*); 1982, on wing-scales of these two; Roonwal, Verma and Thakur (1979, *H. gertrudae* and *H. indicola*), and Roonwal (1981, *H. longiceps*; and 1983b, summary). Papillae (both finger-shaped and thorny) and micrasters (both 0- to 6-armed non-asteroids and 5- to 8-armed asteroids) are present, the former being more numerous.

Heterotermes indicola (Wasmann)
(figure 1; and plates 1 and 2)
Imagoes from Jodhpur (Rajasthan, India)

SEM studies were made on wing membranes, especially in the middle region where the structures are 'typical'. Sculpturing here consists of micrasters composed largely of leafy, V-shaped bodies, together with some 5- to 7-armed asteroid elements and, rarely, of single, unarmed rods. They are arranged in curves (circles, ovoids and spirals, with one or more micrasters at the centre, plate 1, figure 1, LM photograph), but the curvatures are weaker than, for example, in *Speculitermes*. The Vs resemble flower-like bodies with a basal, leafy, thin-walled sheath, with 0 to 3 central, pistil-like arms or projections sprouting from the inner angle of the sheath, but all parts rest basally on the wing surfaces. The arms are often visible through the thin sheath-material; they are generally solid-looking and rod-like, but sometimes they are leafy with pointed tips. Occasionally, the multi-armed Vs are modified into shapes which are intermediate between the Vs and the asteroids, suggesting that the latter probably evolved from the former. The apex of the Vs is generally directed obliquely downward, but the asteroids exhibit no particular orientation. The approximate proportions of the various types are as follows:--

1. Simple rods (rare)	0.2%
2. Simple V, without arm	5%
3. V with 1 arm	18%
4. V with 2 arms	67%
5. V with 3 arms	3%
6. Asteroid with 5 arms	2%
7. Asteroid with 6 arms	4%
8. Asteroid with 7 arms	0.8%

Sizes (length × width) vary as follows:- Rods 6 × 1 μm; Vs 5–7 × 4–7 μm; asteroids (diameter) 4–6 μm. Densities are ca. 9800/mm^2. These are higher than those given earlier by Roonwal and Rathore (1977, ca. 5330/mm^2); the latter may have been underestimates.

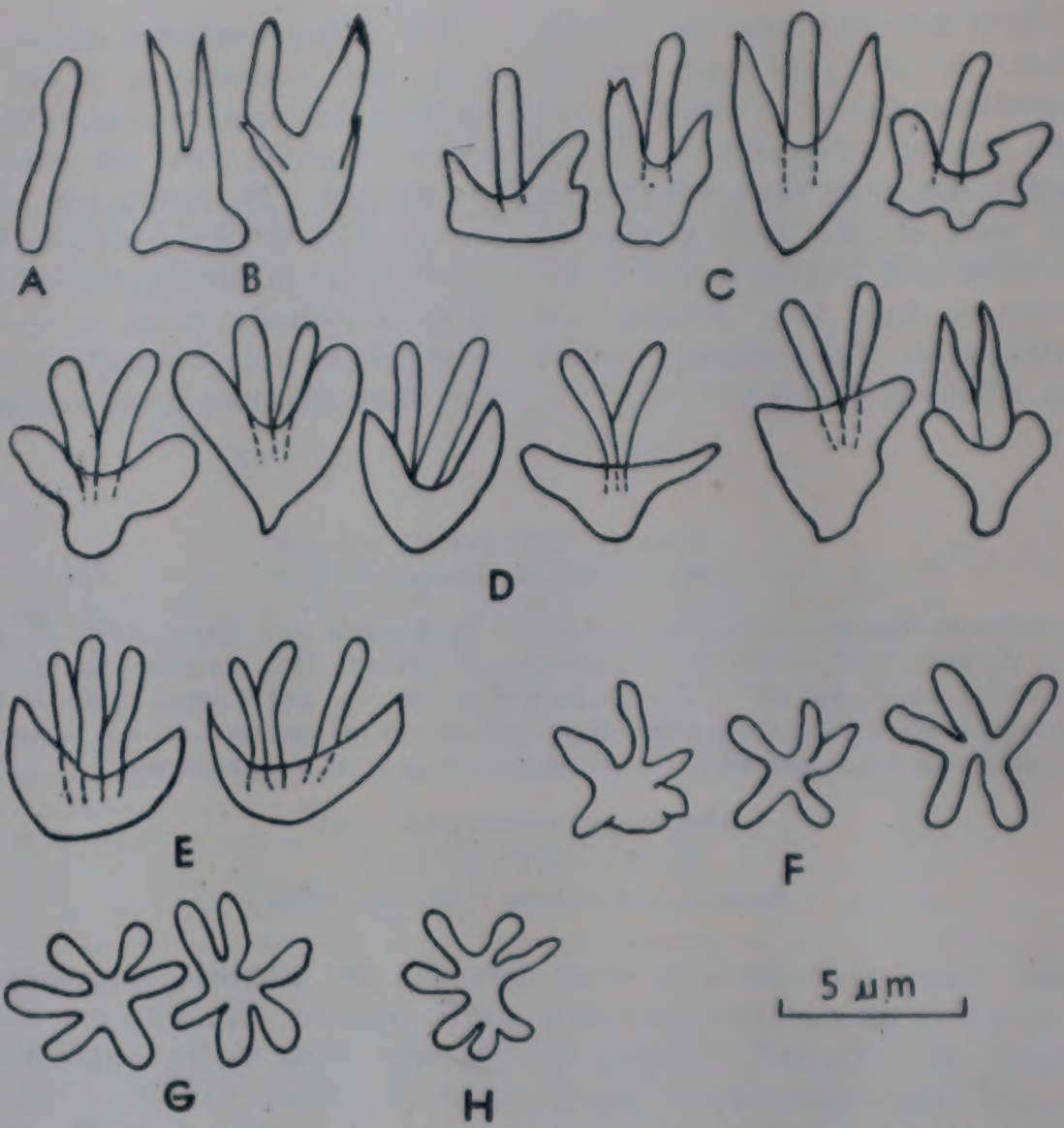

Figure 1 *Heterotermes indicola.* Types of micrasters (from scanning electron micrographs) from dorsal surface of forewing in middle region. **A**, Rod-like (very rare); **B–E**, V-shaped and leafy; **F–H**, Asteroids. (*B*, Vs without central arm; *C*, with an arm sprouting out; *D*, with 2 arms; *E*. with 3 arms; *F*, asteroid with 5 arms; *G*, with 6 arms, and *H*, with 7 arms)

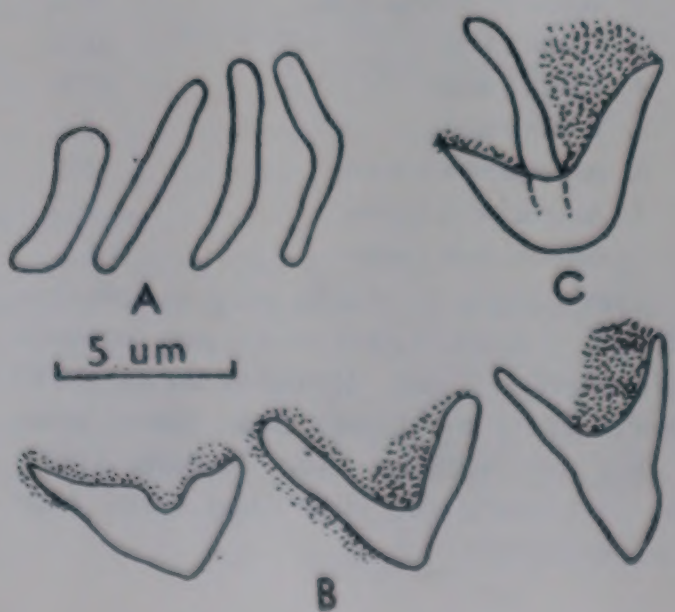

Figure 2 *Microtermes obesi.* Types of simple micrasters (from scanning electron micrographs) from dorsal surface of forewing in middle region. **A**, Simple rods; **B**, V-shaped, leafy, sheath-like, without central arm; **C**, Same, with one arm. Edges of sheaths often projected into thin, irregular, gauze-like extensions

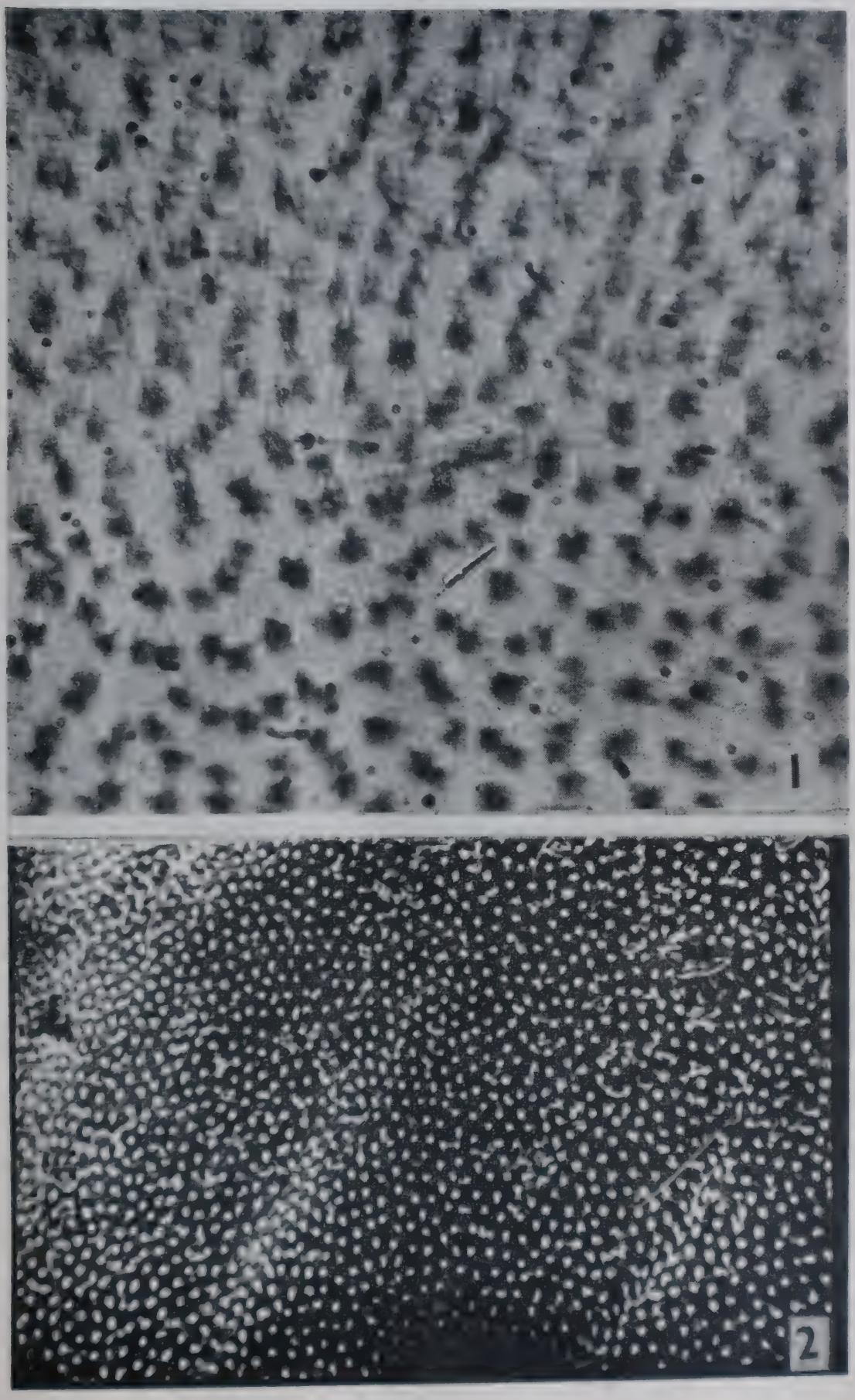

Plate 1 *Heterotermes indicola*. Dorsal surface of forewing membrane in middle, showing micrasters 1, Light microscope photograph (720); 2, Scanning electron micrograph (240)

Plate 2 *Heterotermes indicola* Scanning electron micrographs of dorsal surface of forewing in middle showing microasters [1 (× 1200); 2 (× 2400)]

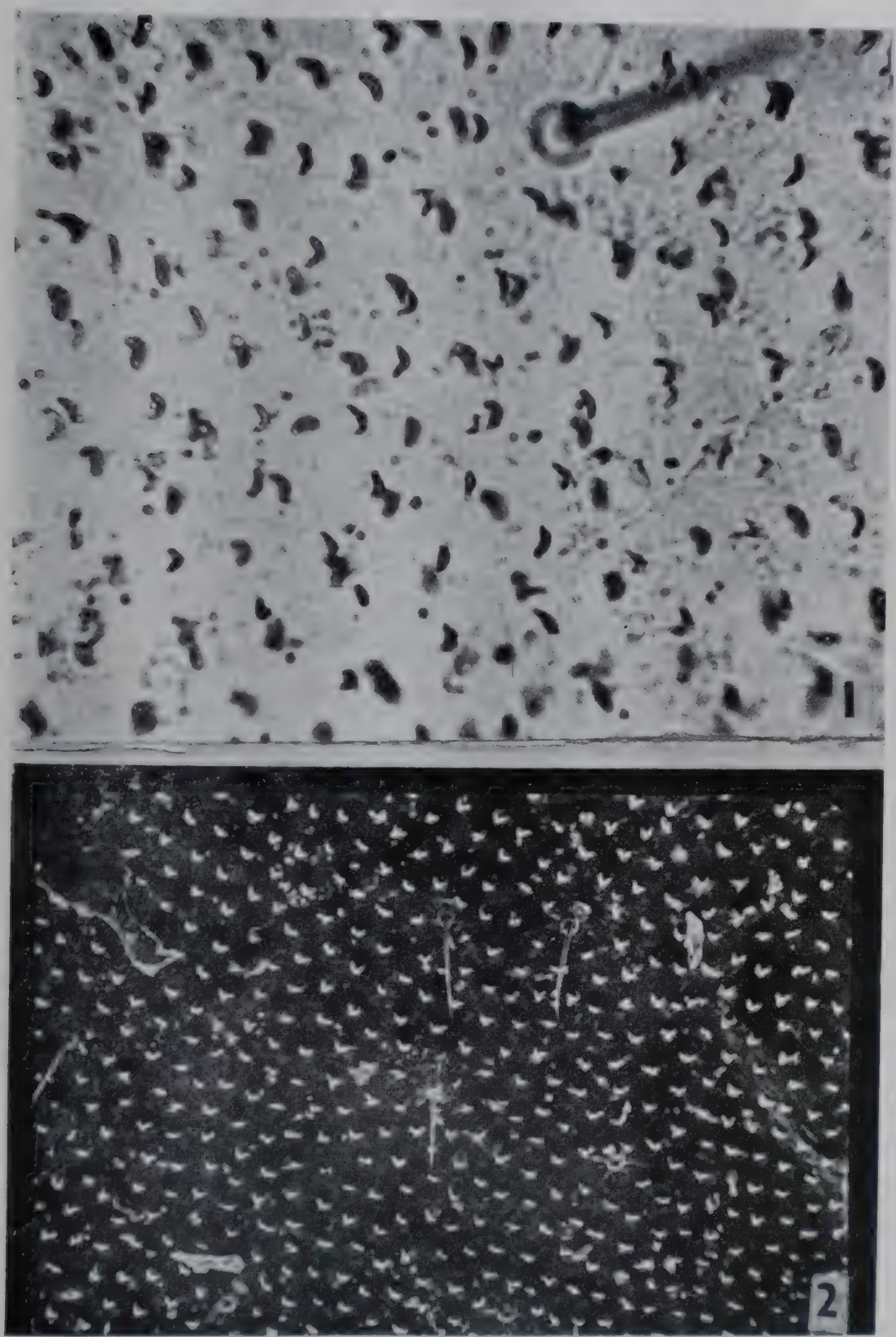

Plate 3 *Microtermes obesi*. Dorsal surface of right forewing in middle, to show micrasters. **1**, Light microscope photograph (× 720). Micrasters of the ventral surface also show through the thin, transparent wing membrane; **2**, Scanning electron micrograph (× 240)

Plate 4 Migratory locust, *Locusta migratoria migratoria* (Linnaeus): 1 and 2, swarms of hoppers in marching formation [1 × 1962; 2 × 1970]

2. Genus MICROTERMES Wasmann
(Termitidae, Macrotermitinae)

LM studies on *Microtermes* have been published by Roonwal and Chhotani (1967, *M. imphalensis, obesi* and *unicolor*) Roonwal, Verma and Rathore (1980, *M. imphalensis, mycophagus, obesi* and *unicolor*) and Roonwal (1983b, summary). Microsculpturing here was regarded as consisting of "rods" in addition to the usual papillae. SEM studies show that the so-called rods in this genus really belong to the micraster series.

Microtermes obesi Holmgren
(Syn. *M. anandi* Holmgren)
(figure 2; and plates 3 and 4)

Imagoes from Jodhpur (Rajasthan, India). For taxonomic position, see Snyder (1949)

SEM studies were made in the middle region of the wing where sculpturing is 'typical' and least modified. Two main types of structures (both classed as micrasters, not 'rods' as stated in the earlier accounts) occur, viz. short, one-armed pieces with round ends and V-shaped. The latter again are of two kinds, viz. simple Vs with a broad or narrow angle, and Vs with a central, pistil-like arm sprouting from the inner angle. The two arms of the V are broad, leafy, thin-walled and sheath-like or flower-like, with the margins often extended irregularly into thin, gauze-like veils. The one-armed micrasters lie somewhat obliquely, while the Vs mostly point obliquely downward. The former are ca. 5–8 μm long; the height of the Vs is ca. 2–7 μm. The structures are distributed uniformly and do not exhibit any curved, circular or spiral pattern as occurs in some termites, e.g. *Heterotermes* (supra), *Speculitermes*, etc. The average density in the middle of wing is 3700/mm^2 (in the earlier accounts, it was recorded as 2644–4759/mm^2). The Vs constitute the bulk (77%) of the sculpturings, the proportions being as follows:

One-armed elements	23%
Simple Vs	70%
Vs with a central arm	7%

Discussion

Heterotermes: In the earlier (LM) studies, both nonasteroid and asteroid micrasters were reported in *H. indicola* and *H. gertrudae*, the asteroids being 5- to 7- armed in the former and 5- to 8- armed in the latter species. In a third species, *H. longiceps* (from Brazil), besides the nonasteroids, only 5-armed elements are present among the asteroids. In the only other known heterotermitine genus, *Reticulitermes* (*R. assamensis*, vide Roonwal 1981), papillae and pimpules alone are present; there are no micrasters, and the genus is, therefore, probably more primitive than *Heterotermes*.

Microtermes: In the earlier (LM) studies the microsculpturings (now regarded distinctly as simple micrasters on the basis of SEM studies), were classed as rods but of a special type, viz. "cuticular rods of *Microtermes*-group", being characterised as "thin, very short and weakly wavy or strongly curved" (Roonwal & Chhotani 1967). They were distinguished from rods of the *Odontotermes*-type which are much thinner, longer and apparently more or less wavy. Similar thick, one-armed, rod-like structures have also been reported in two other macrotermitine genera, viz. *Allodontermes* Silvestri (Roonwal et al. 1980) and *Ancistrotermes* Silvestri (Roonwal 1981), but were interpreted as simple, nonasteroid micrasters. The intimate structure of the so-called rods, as now revealed by SEM studies in *Microtermes*, make it clear that they should be classed not as rods but as simple micrasters

of the nonasteroid type (of Roonwal et al. 1974). In another macrotermitine genus, *Pseudacanthotermes*, neither rods nor simple micrasters but microsetae occur (Roonwal et al. 1980). These considerable differences between the various macrotermitine genera would suggest that the subfamily Macrotermitinae is a heterogeneous assemblage of phylogenetically diverse genera, and a careful re-study of the subfamily would be profitable.

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Ultrastructural Changes in the Median Neurosecretory Cells of *Spodoptera litura* (Fabricius) Infected with a Baculovirus

B SUBRAHMANYAM[1], N RAMAKRISHNAN[1] and NAM PRAKASH[2]
Division of Entomology[1], *Nuclear Research Laboratory*[2], *Indian Agricultural Research Institute New Delhi 110 012*

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Ultrastructure of the median neurosecretory cells (MNC) of the larvae of tobacco leaf-eating caterpillar, *Spodoptera litura*, that fail to undergo larval pupal moult, following a baculovirus (nuclear polyhedrosis virus) infection is described. Larvae of the penultimate instar were fed with a dose of 5.57×10^6 polyhedra/larva and the ultrastructural changes of the MNC were studied from the appearance of external symptoms until the period of lethal infection. Concentric arrays of rough endoplasmic reticulum and enhanced production of neurosecretion suggest hypertrophy of the MNC of the infected larvae. Virus multiplication and polyhedral formation are restricted to glial and tracheal cells in the *pars intercerebralis*. The nucleus of MNC is not a site of virus replication. The implications of virus infection on the activity of MNC are discussed.

Key Words: *Spodoptera litura*, **Nuclear polyhedrosis virus, Ultrastructure, Median neurosecretory cells**

Introduction

Interaction of virus with the host occurs at molecular level which results in morphological, histological and biochemical changes in the host. Obviously, these changes favour the replication of the virus in the host tissues. Morphogenetic changes like prothetely in nuclear polyhedrosis virus (NPV) infected western oak looper, *Lambdina fiscellaria somniaria* and tussock moth, *Orgyia pseudotsugata* (Morris 1970) and metathetely due to entomopox virus infection of *Choristoneura biennis* (Retnakaran & Bird 1972) were reported earlier. The tobacco leaf eating caterpillar, *Spodoptera litura* (Fabricius) afflicted with NPV fails to pupate and attains an abnormal size (Komolpith 1974). Further, Subrahmanyam and Ramakrishnan (1981) showed that after NPV infection, *S. litura* moults only once and that the aldehyde fuchsin stainable material in the median neurosecretory cells (MNC) increases during disease development. It was also shown that the failure of the diseased larvae to undergo larval pupal moult is due to maintenance of juvenile hormone titre in the haemolymph even at the later part of last

instar (Subrahmanyam & Ramakrishnan 1980). In this paper we describe the changes observed in the ultrastructure of the median neurosecretory cells of the virus infected larvae. Ultrastructural features of the MNC of last instar of the healthy larvae were described in our earlier publication (Subrahmanyam et al. 1981).

Materials and Methods

Laboratory culture of *S. litura* was maintained at 27±2°C and 75% RH according to Pawar and Ramakrishnan (1971). Test larvae of the same instar and age were obtained, following the moulting process. They were reared individually from the fourth instar. Each one of the infected larvae received an aquous suspension of virus (5.57×10^6 polyhedra) evenly dispensed on a circular leaf disc, a day prior to the last larval moult. This dose was found, in a preliminary test, to be optimum for causing a lethal infection. The brain tissue was daily processed for ultrastructure study, from the first day of the last instar till the period of lethal infection. The brain tissue was fixed in 6.25% glutaraldehyde, post-fixed in osmium tetroxide and embedded in Epon-Araldite mixture as per Subrahmanyam et al. (1981). Ultrathin sections (300–400Å) were cut on an LKB microtome (Model 4801), stained with uranyl acetate and lead citrate and examined under transmission electron microscope (Philips EM 300) operated at 60 KV.

Results

Symptoms of polyhedrosis appeared three days after infection. At this stage the larvae were 11-day old and in the 6th inster. The diseased larvae failed to pupate on the 13th day and died after 48 hr. Changes in the fine structure of the MNC were studied from the onset of symptoms till the period of lethal infection.

The secretory cells that were almost devoid of neurosecretory droplets (NSD) on the 11th day were found to synthesize neurosecretion from the 12th day onwards. This is evidenced by the sparsely distributed NSD in the MNC on the 12th day. The conspicuous changes observed at this stage are (*i*) aggregation of mitochondria near the nuclear membrane and (*ii*) vacuolation of the cytoplasm. Mitochondria are small, irregularly oval and cristae are almost fused. Ribosomes are free and present in small aggregations (figures 1 and 2).

With the pronounced appearance of external symptoms on the 13th day, the activity of MNC appeared to increase as evidenced by the increase in the neurosecretion, appearance of rough endoplasmic reticulum (RER) and abundance of ribosomes. Associated structural changes are (*i*) disorganised golgi apparatus (GA that lack the cisternae), (*ii*) extensive vacuolation, and (*iii*) fusing of adjacent mitochondria. Ribosomes have dispersed on the surface of endoplasmic reticulum appearing as an extensive network (figures 3 and 4).

On the ultimate day of lethal infection also, the neurosecretory cells showed pronounced activity. The micrographs 5 to 8 show the nucleus having dispersed chromatin and the cytoplasm having profuse NSD. The RER is extensively dispersed in concentric parallel arrays not only around the nuclear membrane but also in the entire cytoplasm. Myelin figures with concentric whorls of electron dense material are prominent in these secretory cells.

The NSD are less electron dense and do not have the characteristic round shape but are irregularly oval often showing distortion of shape. The membrane envelopes of these droplets are not clear. Some of the NSD are devoid of this envelope while others have incomplete envelopes. The diameter of these NSD ranged from 1050-1870 Å.

Though there are characteristic changes in the activity of MNC during the course of disease development, neither free virions nor

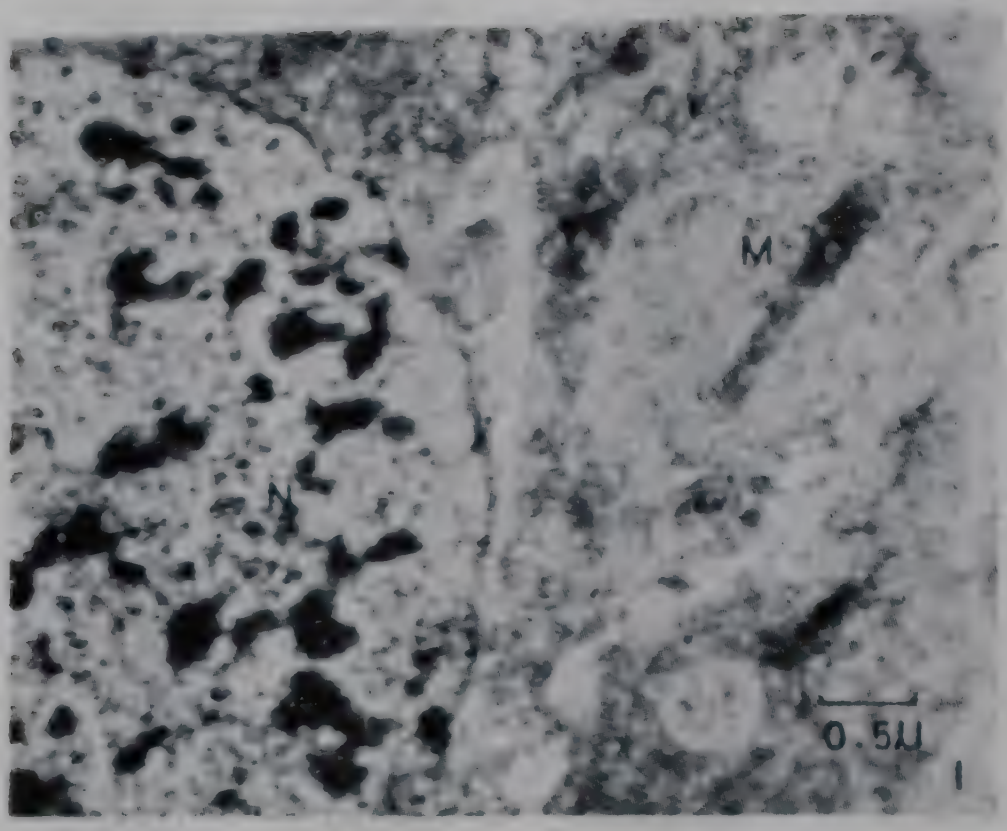
polyhedral protein matrix was noticed in the nuclei of the secretory cells. However, certain histopathological changes occur in the glial cells and associated neurones of the *pars intercerebralis*. The nuclei of the nerve cells adjoining the MNC have aggregation of chromatin, and cytoplasm has elongated mitochondria whose cristae are totally disorganised (figure 9). The glial cells that surround MNC have fibrous material similar to that of polyhedral matrix (figure 10). Incorporation of virus particles into polyhedral matrix is noticed in the adjoining tracheal and glial cells (figure 11).

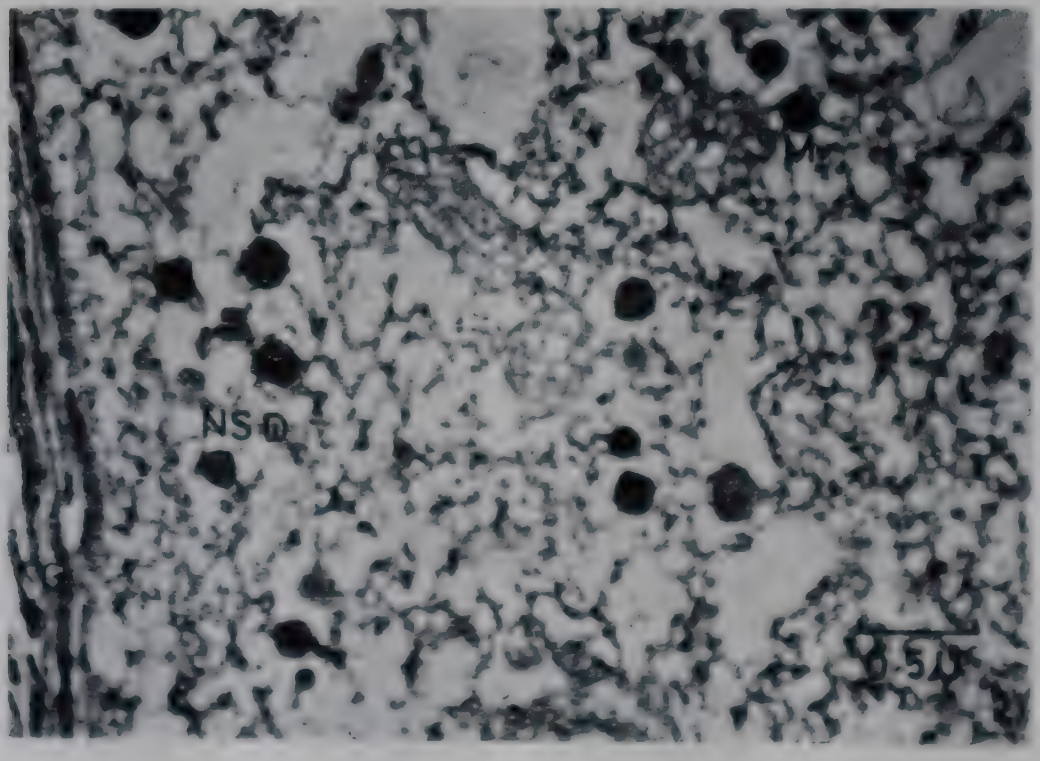
Discussion

During the course of the disease, neither free virions nor polyhedral formation was noticed in the nuclei of MNC. Pawar (1974) in his study on the histopathology and development of virus in NPV infected *S. litura*, had observed only moderate infection of abdominal ganglia. Virus particles seen were largely contained in vesicles in neural lamella and cytoplasm of glial cells. Nuclei of glial cells were seen having virus particles occluded in amorphous polyhedral protein. But the axons in the synaptic area were not infected. In the present study we noticed the polyhedral matrix only in the glial processes and tracheae of the brain.

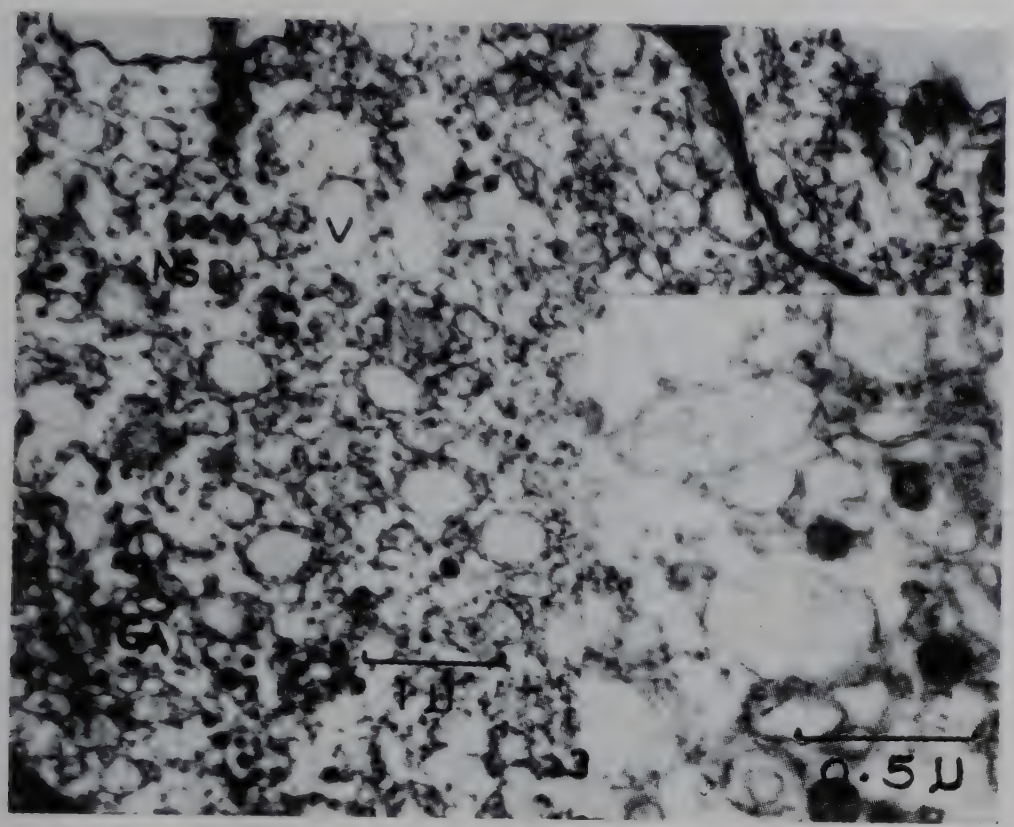
It is of particular interest to notice several pathological changes in the ultrastructure of MNC leading to altered secretory activity. During the initial stage of polyhedrosis the secretory activity was very low but with disease advancement, the MNC were packed with NSD. This phenomenal increase in neurosecretion observed in the diseased larvae coincides with the deep staining of MNC with paraldehyde fuchsin already reported by Subrahmanyam and Ramakrishnan (1981). Applying the same staining technique to epoxy sections, it was shown by Steel and Morris (1977) that secretory granules account for much of the affinity of NSC for aldehyde fuchsin. In addition, the cytoplasm of the MNC contain parallel arrays of RER almost in a concentric fashion. Such concentric lamellar bodies derived from the endoplasmic reticulum and usually composed of paired membranes have been observed in some normal cells and a variety of pathologically altered cells, especially viral hepatitis in man and mouse (Ghadially 1975). It is by now well established that RER is involved in the synthesis of proteinaceous neurosecretion in insects (Borg & Marks 1973, Donae 1973, Gilbert & King 1973, Slama et al. 1974, Steel 1981). Benz (1963), postulated that metabolic activity, especially protein synthesis, is highly stimulated in the infected cells. Pawar and Ramakrishnan (1977) reported a significant change in the total haemolymph proteins of NPV infected *S. litura* larvae. These suggest a general influence of polyhedrosis on protein synthesis leading to formation of polyhedral and viral proteins. Our study on the other hand presents evidences suggestive of the enhancement of secretory protein synthesis in MNC. Increase in the amount of RER, a state of altered functional activity, resulting in an increase in the seecretory protein of MNC is an indication of hypertrophy at the advanced stage of polyhedrosis. in the cells that are refractory to virus multiplication, like MNC, only the host protein synthesis is accelerated while the susceptible cells are programmed to synthesize viral protein by the alteration of the host genetic code at the transcriptional level.

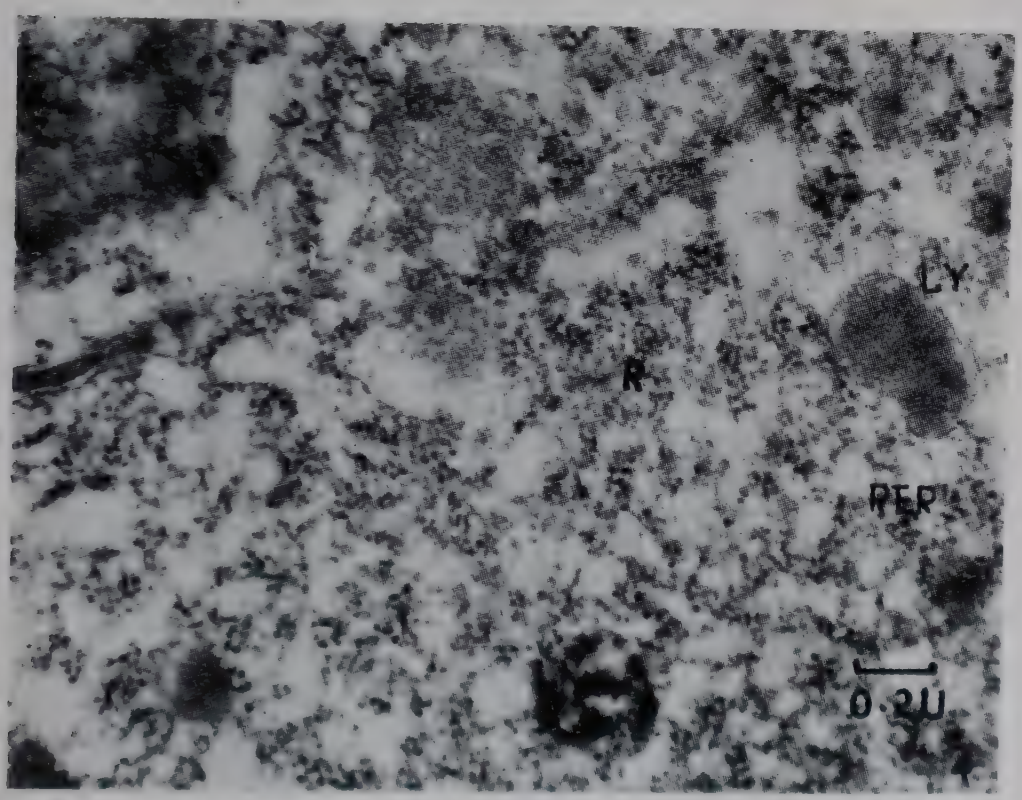
The NSD observed in the MNC from healthy *S. litura* larvae are electron dense, round and membrane-bound measuring 2100-2600$\mathring{A}$ (Subrahmanyam et al. 1981). In the MNC of 14-days old diseased larvae NSD are seen to have lost their characteristic round shape and the limiting membrane is either incomplete or loosely bound (figure 12). Golgi complex is associated in the final packing of the secretory product into membrane



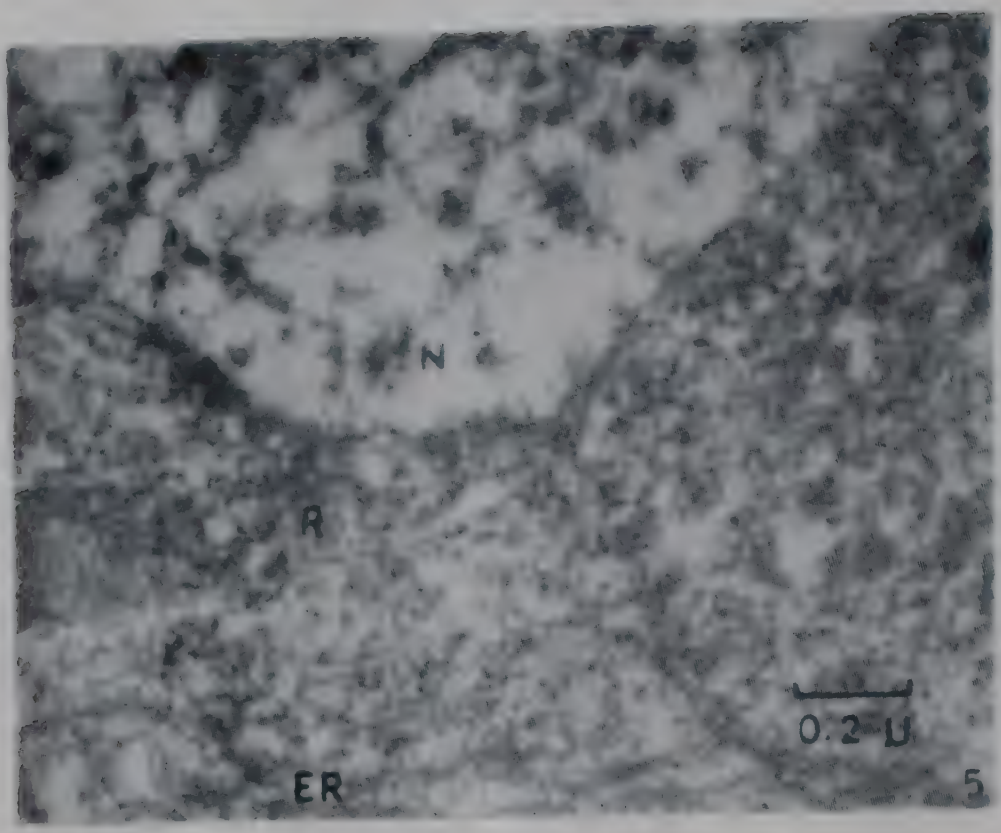


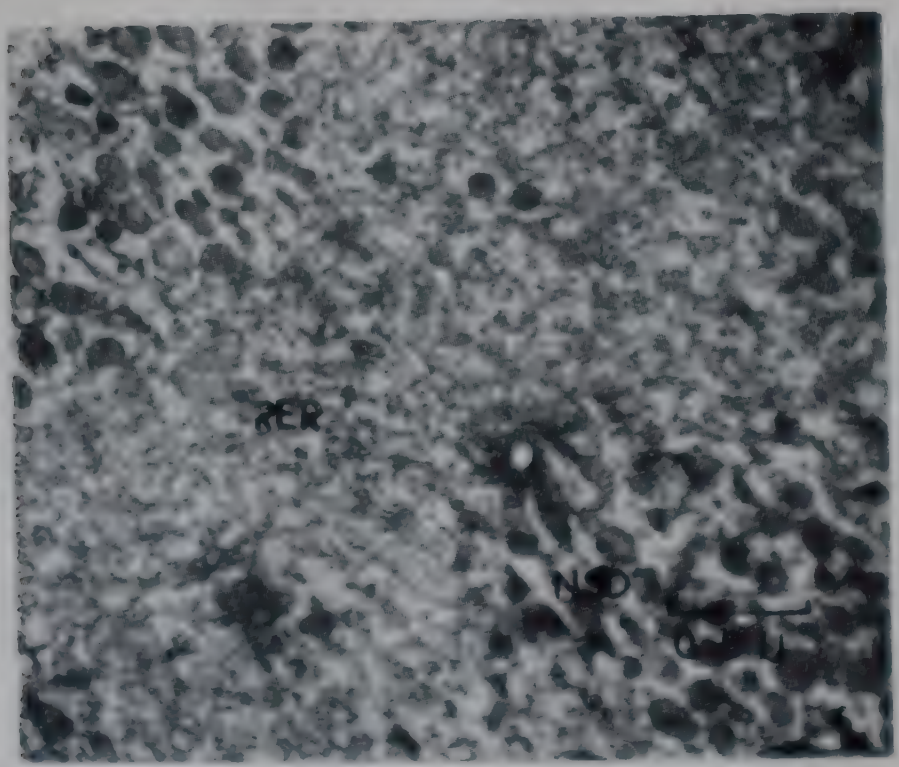
Figures 1 and 2 MNC of NPV infected 12-day-old larvae 1 aggregation of mitochondria (M), and 2 sparsely distributed NSD in cytoplasm



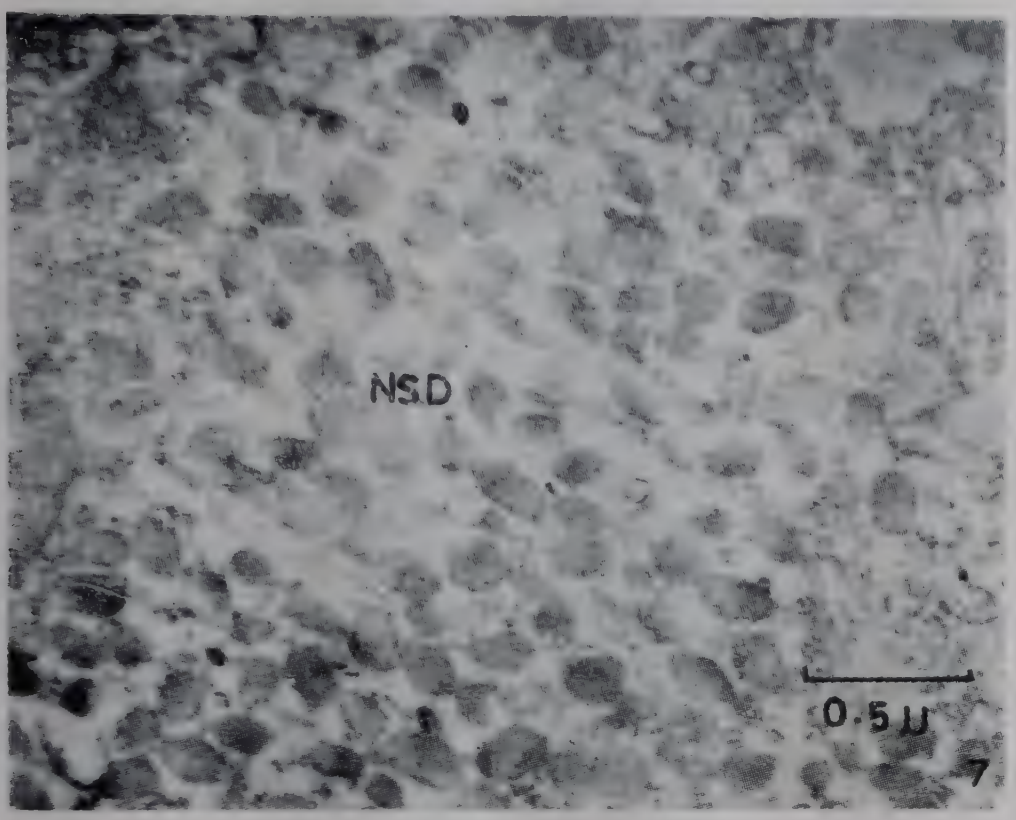


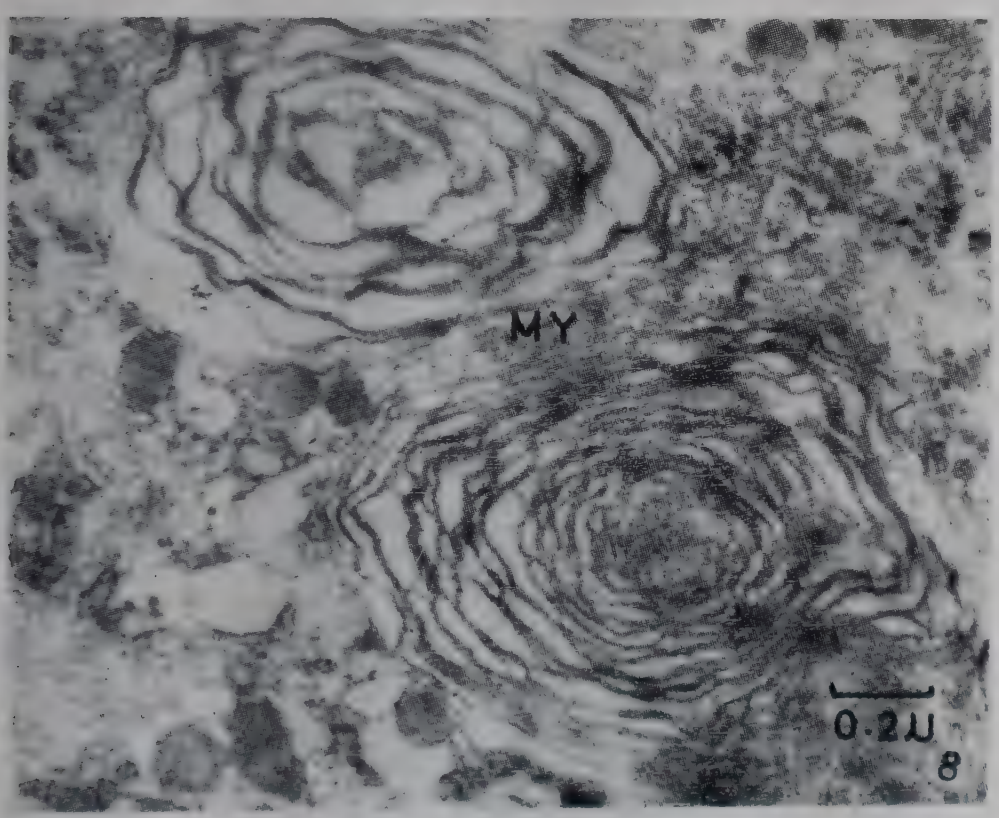
Figures 3 and 4 MNC of NPV-infected 13-day-old larvae. **3**. Golgi apparatus (Inset enlarged GA), vacuole in cytoplasm (**V**); **4**. area enlarged showing abundant ribosome (**R**), formation of RER and Lysosomes (**Ly**)





Figures 5 to 8 showing marked increase in secretory activity of MNC of 14-day-old larva. Note the appearance of parallel arrays of RER, abundant NSD, whorls of myelin figures (My) in the cytoplasm and dispersed chromatin in the nucleus (N)


NSD
0.5 μ
7


MY
0.2 μ
8

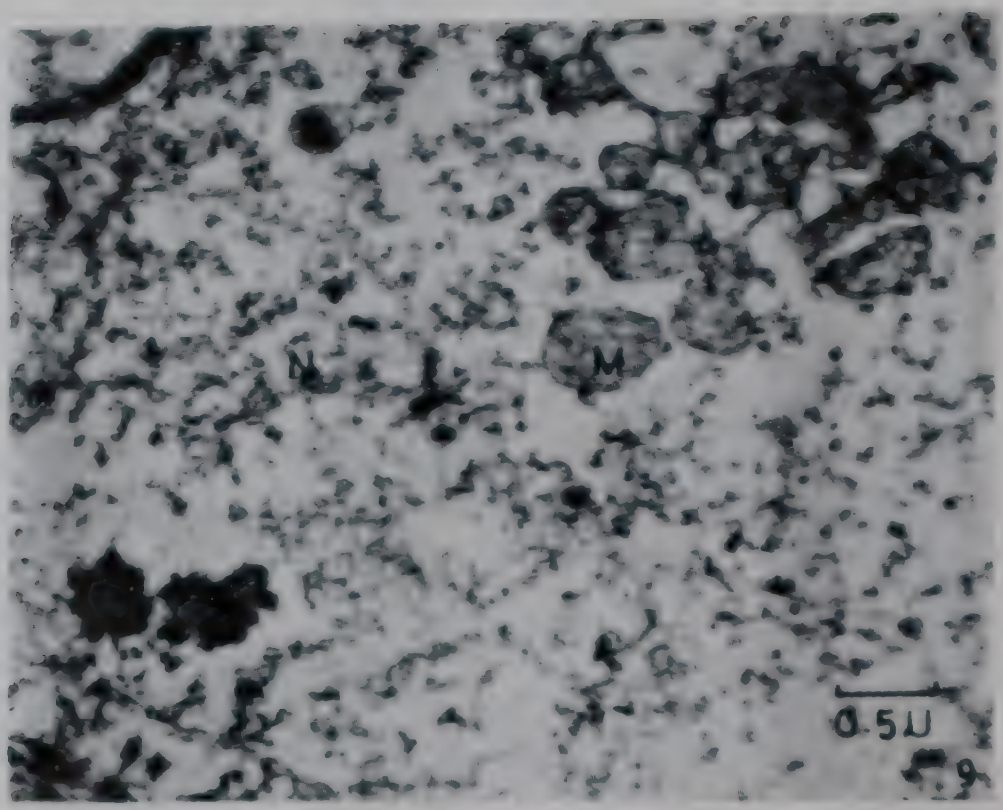


Figure 9 A neuron having aggregation of chromatin in the nucleus and disintegrations of mitochondria

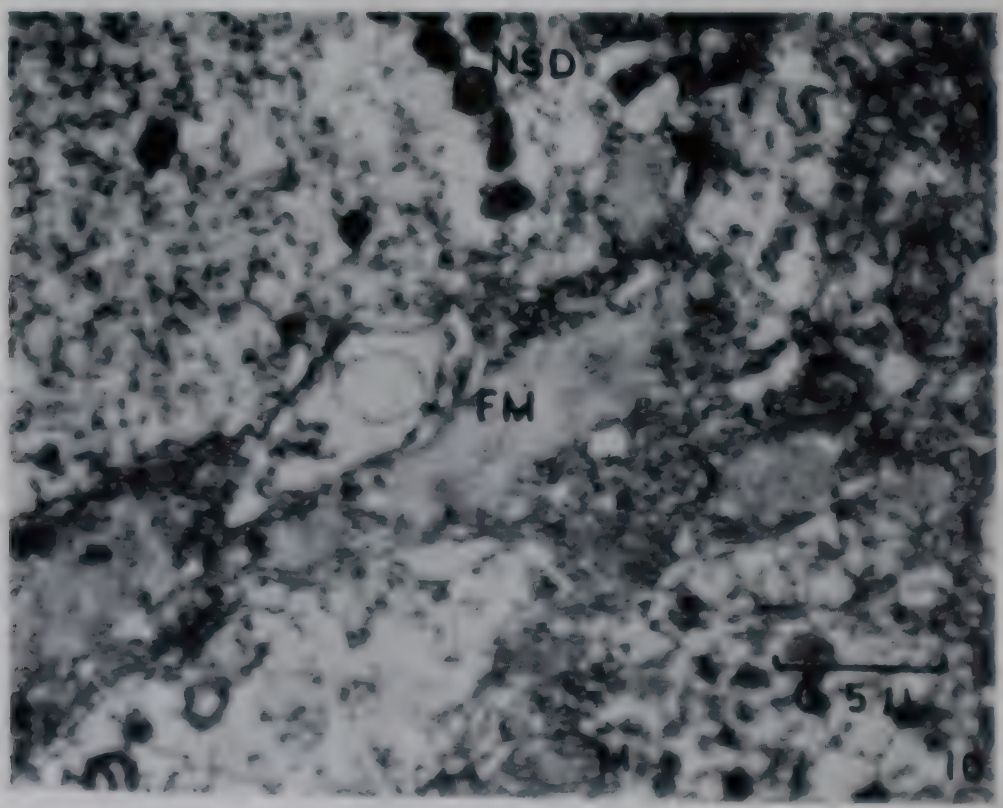


Figure 10 formation of fibrous material (FM) of polyhedra exterior to the secretory cell in glial processes

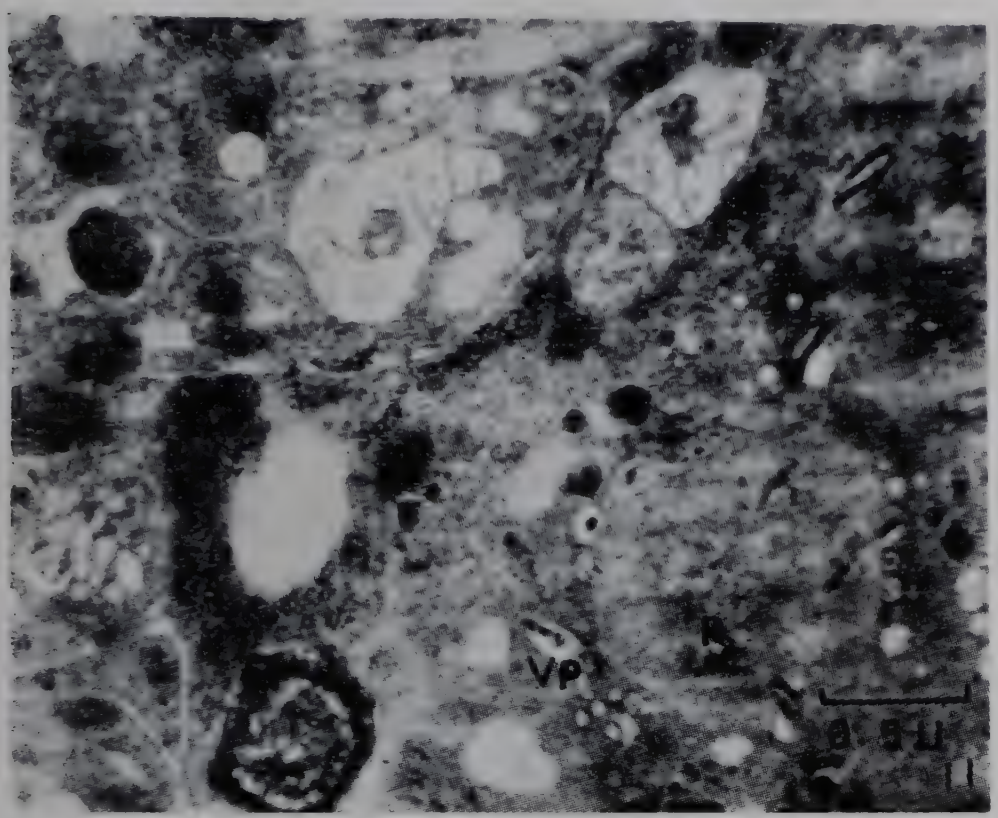


Figure 11 Embedding of virus particles (**VP**) into the polyhedron (**P**) in the glial cell nucleus

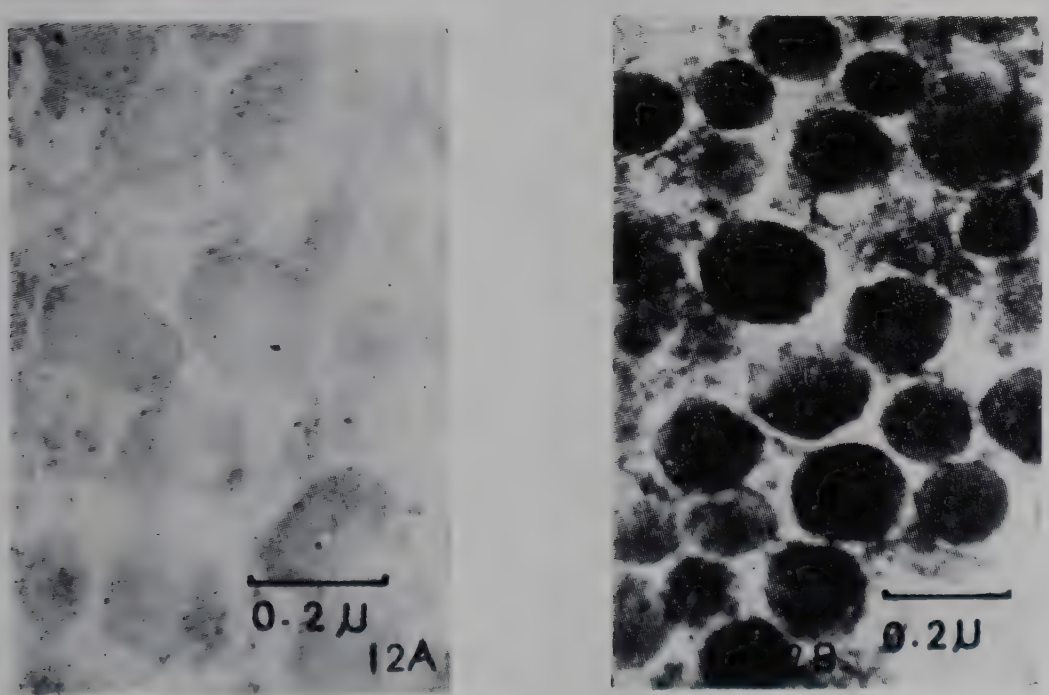


Figure 12 NSD from diseased (**A**) and healthy larvae (**B**)

bound vesicles (Fawcett 1966, Doane 1973). The GA observed in the present study is lacking cisternae suggesting its dysfunction leading to the observed variations of the granule morphology.

Osmiophilic whorls of mylein figures in the cytoplasm of MNC of diseased larvae indicate the initiation of degenerative process as these represent lysosomal bodies containing degradative enzymes. Such mylein bodies were identified in the herpes virus infected cells, rat sarcoma and human ovarian sarcoma (Ghadially 1975), neurones of cases of Tay-sach disease (Terry & Weiss 1963) and in the tracheal cells of NPV infected *S. litura* (Pawar 1974). The mitrochrondria were found in aggregations in the MNC as well as in adjacent neurones and giant mitochondria were formed probably by enlargement and fusion of the membranes of several adjacent mitochondria.

This study supports the deep staining of MNC of diseased larvae with aldehyde fuch sin reported earlier by us signifying the abundance of neurosecretion and also presents various pathologically induced ultra structural changes.

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Histochemical and Biochemical Studies of Myotomal Muscle Types in the Pre-adult and Adult Freshwater Carp, *Labeo rohita* (Hamilton)

SHASHI KIRAN and C L TALESARA*
Muscle Physiology and Histochemistry Unit, Department of Zoology, University of Delhi, Delhi 110007

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Three discrete types of muscles—red, white and pink have been recognized in the myotomal musculature of pre-adult and adult fresh water teleost fish *Labeo rohita*. A detailed histochemical and biochemical *p*H-profile for the enzyme m-ATPase was undertaken. Both red and white muscles, have shown characteristics similar to those found in other teleosts. These are classified as slow twitch red-type I and fast twitch white-type IIB, respectively. The pink fibres are distinguished as a separate class with distinctive histochemical and biochemical characteristics and can be classified as fast twitch red-type IIA or C fibres.

Key Words: Myotomal muscles, Myofibrillar-ATPase, Histochemical profile, Freshwater carp

Introduction

Most of the teleost fishes possess two main types of myotomal (Boddeke et al. 1959, Bone 1966, Johnston et al. 1972) as well as respiratory (Ojha 1975, Munshi et al. 1975, Hughes & Johnston 1979) muscles.

However, the myotomal musculature of some teleosts have more than two muscle types (Johnston et al. 1974, Patterson et al. 1975, Bone 1978, Johnston 1981) and apparantly they overlap in function between these different types. The most common fibre type found in addition to red and white is the 'pink' or 'intermediate' situated between the red and white muscle forming a narrow zone of about 3-4 layers of fibres (Johnston et al. 1977, Carpene et al. 1982, Talesara & Shashi Kiran 1982, 1983, Shashi Kiran & Talesara 1984). In the mirror carp these comprise around 1% of the myotomal bulk making them slightly more numerous than the red fibres (Johnston et al. 1977). Little is known about the contractile properties, metabolism or role of these pink fibres in the myotomal muscles probably because of their usual presence in minor proportions.

Additionally, the structure and function of adult teleost propulsive musculature have been the subject of much debate but not much

*Address for correspondence

attention has been given to the types of muscle fibres in the pre-adult stages. As the different fibre types of the adult fish have been assigned different roles in swimming, the types of fibres present in the pre-adult stages would also have the characteristics to suit the swimming behaviour shown by immature fishes (Proctor et al. 1980).

The present work was undertaken to determine the muscle fibre types in the adult and pre-adult stages of the fresh water teleost *Labeo rohita*.

Materials and Methods

Fish, rohu carp, *Labeo rohita* (Hamilton) were locally collected. Muscle samples were excised from the trunk as well as from the caudal myotomal regions. Small muscle pieces were mounted on cryostat chucks and quickly frozen by immersion in liquid nitrogen. The blocks were placed in the cryostat (IEC-Model CTD) at −25°C. The frozen sections of 10 μm thickness were cut and used for histochemical studies.

Histochemical Methods

Myofibrillar-ATPase (m-ATPase) and succinic dehydrogenase (SDH): Frozen sections were air dried for 60–90 min. and stained using routine m-ATPase method of Padykula and Herman (1955) after preincubating the sections in acid or alkali medium as described by Guth and Samaha (1970). The preincubation period varied from 5 sec to 2 min. The incubation was carried out in every case at 37°C, *p*H 9.4 for about 15 min. The *p*H-profile for m-ATPase staining was done under the same conditions as above at a serial range from *p*H 4.35–10.4 with varying preincubation periods. For the enzyme SDH fresh frozen sections of the muscle were incubated for 15–20 min in the medium described by Nachlas et al. (1957) using Nitro-BT as an electron acceptor.

Biochemical Assays

m-ATPase and SDH: Myofibrils were prepared according to the method of Perry and Grey (1956). The reaction for m-ATPase was carried out at *p*H 7.5 and the detailed procedure was the same as reported earlier (Talesara & Goldspink 1978, Talesara & Shashi Kiran 1982). *p*H-profile for Ca^{++}-activated ATPase enzyme assay was performed on the myofibrils prepared from various muscles under the same assay conditions as described above at a serial range of *p*H from 4.35 to 10.4 in accordance with the work reported earlier (Talesara & Goldspink 1978, Talesara & Shashi Kiran 1982, 1983). The assay procedure for SDH was essentially based on the method given by Nachlas et al. (1960) using INT [2 (p-Iodophenyl) 3-(p-nitrophenyl)-5-phenyl-tetrazolium chloride] as the artificial electron acceptor. Protein concentration was determined by the biuret method (Gornall et al. 1949). All the values for m-ATPase and SDH were expressed as μ moles Pi liberated mg^{-1} protein min^{-1} and μg formazan mg^{-1} protein min^{-1} respectively.

Results

Adult Muscle Fibres

In the myotomal musculature of the fish *Labeo rohita*, three different muscle types could be recognized macroscopically (figure 1).

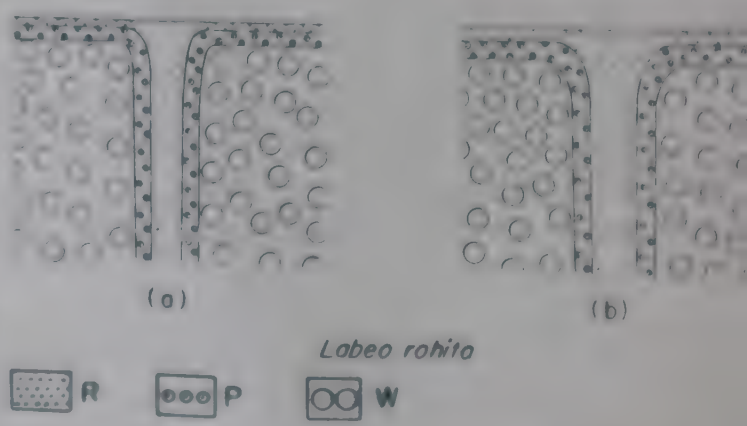


Figure 1 Diagram (reproduced from figures 2–8) showing the distribution and relaive abundance of various muscle types in the trunk (*a*) and tail (*b*) myotomal muscles of the adult fresh water teleost *Labeo rohita* (R, Red; W, White; P, Pink)

These were seen to have different characteristics by histochemical and biochemical studies.

Histochemically, the three types of muscles (red, white & pink) fibres could be easily distinguished in the adult *Labeo rohita* by their SDH activities. The activity of this enzyme was quite high in all the superficial fibres. The intermediate part also showed some degree of staining whereas the muscle fibres of the deep part had very low SDH activity (figures 2 and 6).

The three muscle fibre types also showed characteristic m-ATPase staining pattern. The alkaline ATPase reactions (preincubation in alkali *p*H) resulted in a dark staining of all the fibre types (figures 3 and 4). In the acidic *p*H-range the staining was very low in the fibre types but increased towards the alkali range (table 1). An increase in *p*H above 10.1–10.2 resulted in complete inactivation of the red fibres (fibures 5, 7 and 13). The next fibres to be inactivated in alkali *p*H were the pink fibres, i.e. the fibres showing intermediate staining of SDH activity (figure 8). The white fibres were the most resistant to alkali *p*H, showing some staining even at 10.4 where all the other fibres are completely inactivated (figure 8 and table 1).

Biochemically, the red muscle showed quite high levels of SDH activity from the trunk as well as tail regions. The pink muscle showed intermediate activity levels while the white muscle had the lowest levels for this enzyme (table 2).

Biochemical levels for the enzyme m-ATPase revealed about 3-4 times higher activity in the white muscles as compared to the red. The activity levels of the pink muscle was found to be intermediate between red and white muscles (table 2). Biochemical *p*H-profile for m-ATPase revealed that the red muscles are the first ones to be inactivated in the alkali *p*H-range as observed by histochemical *p*H-profile. The pink and white

Table 1 *Histochemical* p*H-profile for m-ATPase in the trunk muscles of the* Labeo rohita

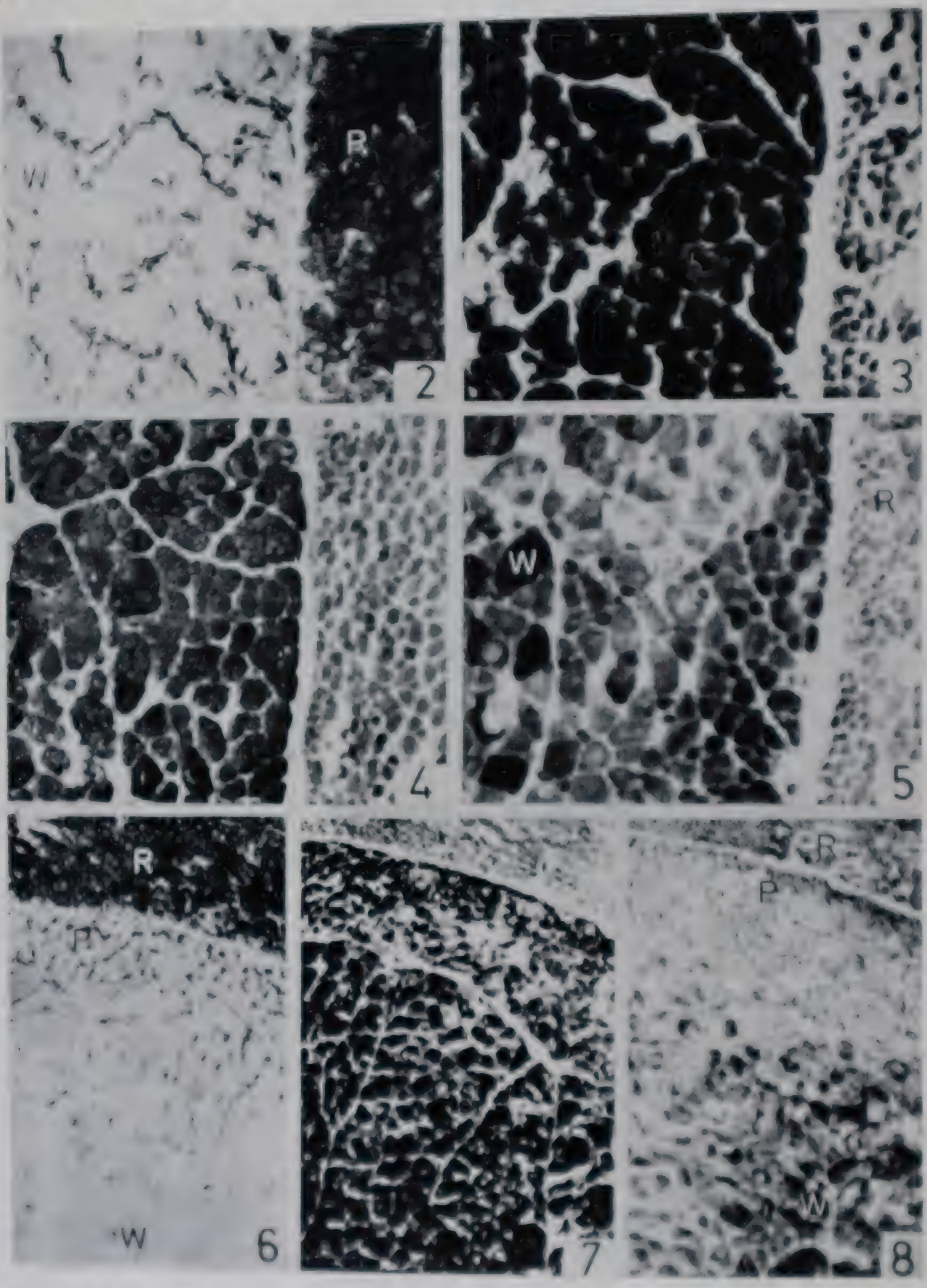
Preincubation		m-ATPase activity		
		Fibre types		
*p*H	Time	Red	Pink	White
4.35–5.5	5–10 sec	±	±	±
5.6–7.9	5–10 sec	1+	1+	1+
8.0–9.6	5–10 sec	1+	2+	2+
9.7–9.8	5–10 sec	2+	3+	3+
9.9–10.0	5–10 sec	3+	4+	4+
10.0	1–2 min	2+	4+	4+
10.1	5–10 sec	2+	4+	4+
10.1	1–2 min	1+	3+	3+
10.2	5–10 sec	±	3+	3+
10.2	1–2 min	—	1+	2+
10.3	5–10 sec	—	—	1+
10.3	1–2 min	—	—	±
10.4	5–10 sec	—	—	±
SDH	—	4+	2+	±

4+, Intensive staining; 3+, Good staining; 2+, Moderate staining; 1+, Poor staining; ±, Traceable staining; —, No staining

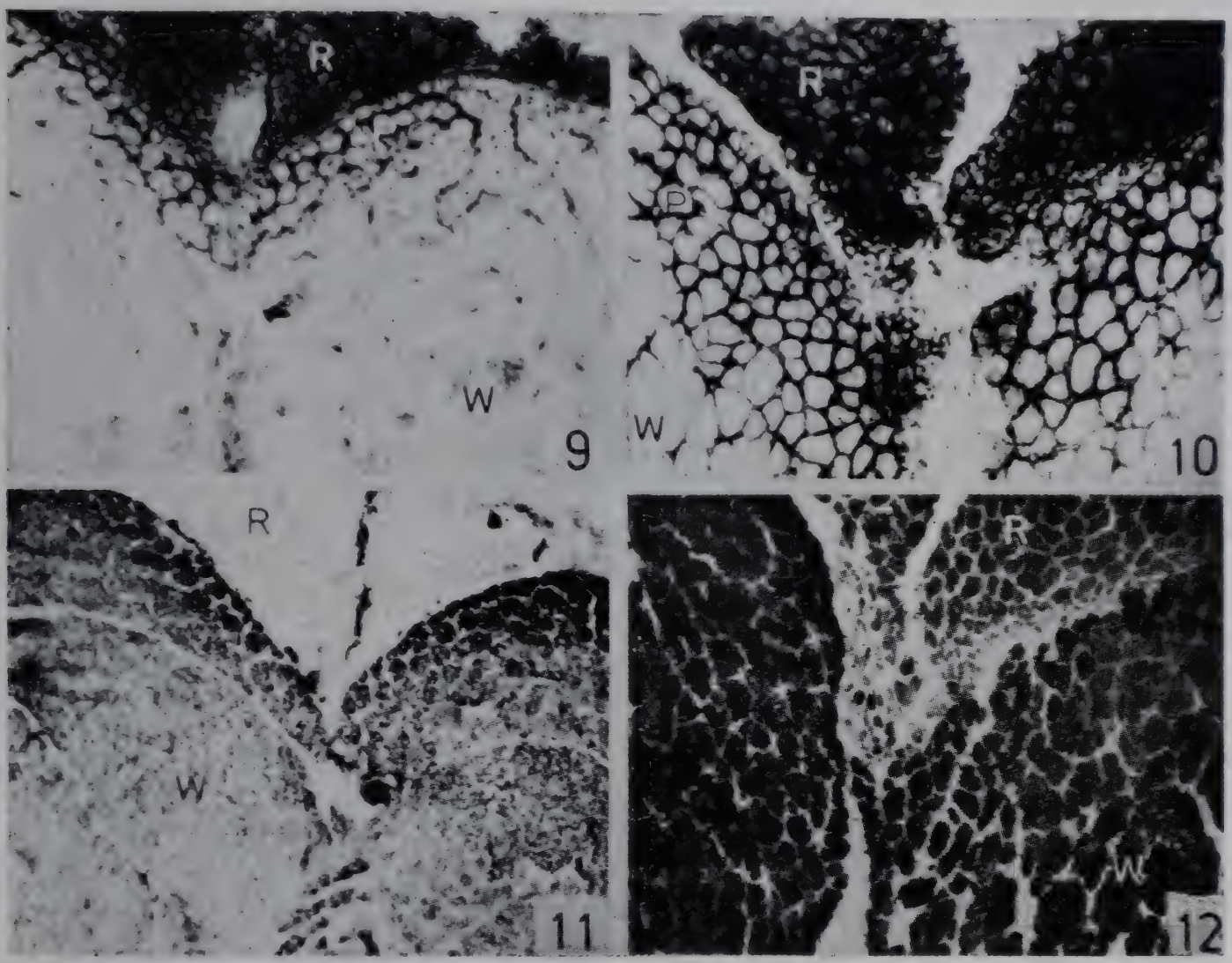
Table 2 *m-ATPase and SDH activity* in the trunk and tail muscles of the fresh water teleost,* Labeo rohita

Particulars	Myotomal region	
	Trunk	Tail
m-ATPase		
Red	0.218±0.011	0.260±0.015
White	0.807±0.020	0.889±0.018
Pink	0.578±0.046	0.686±0.029
SDH		
Red	2.221±0.217	2.176±0.136
White	0.560±0.049	0.682±0.064
Pink	1.092±0.232	1.022±0.080

*Values are Mean ± SE
m-ATPase : μ moles Pi. mg^{-1} protein. min^{-1}
SDH: μg formazan. mg^{-1} protein. min^{-1}



Figures 2-8 Transverse sections of the myotomal muscles of adult *Labeo rohita* showing Red (R), White (W) and Pink (P) fibres. 2-5, Trunk muscles (× 120); 6-8, Tail muscles (× 45). 2 and 6 stained for SDH; 3-5 and 7-8, Incubated for m-ATPase after varying preincubation (PI) *p*H and time: 3, 10.0 (5-10 sec); 4, 10.1 (1-2 min); 5 & 7, 10.2 (1-2 min) and 10.3 (5-10 sec), respectively; 8, 10.4 (5-10 sec).



Figures 9–12 Transverse sections of the myotomal muscles of pre-adult (**9 & 11**, 11/2 month) and (**10 & 12**, $3\frac{1}{2}$ months) *Labeo rohita* showing Red (**R**), White (**W**) and Pink (**P**) fibres (× 120). **9 & 10**, stained for SDH; **11 & 12**, Incubated for m-ATPase at *PI-p*H 10 0 (5–10 sec.)

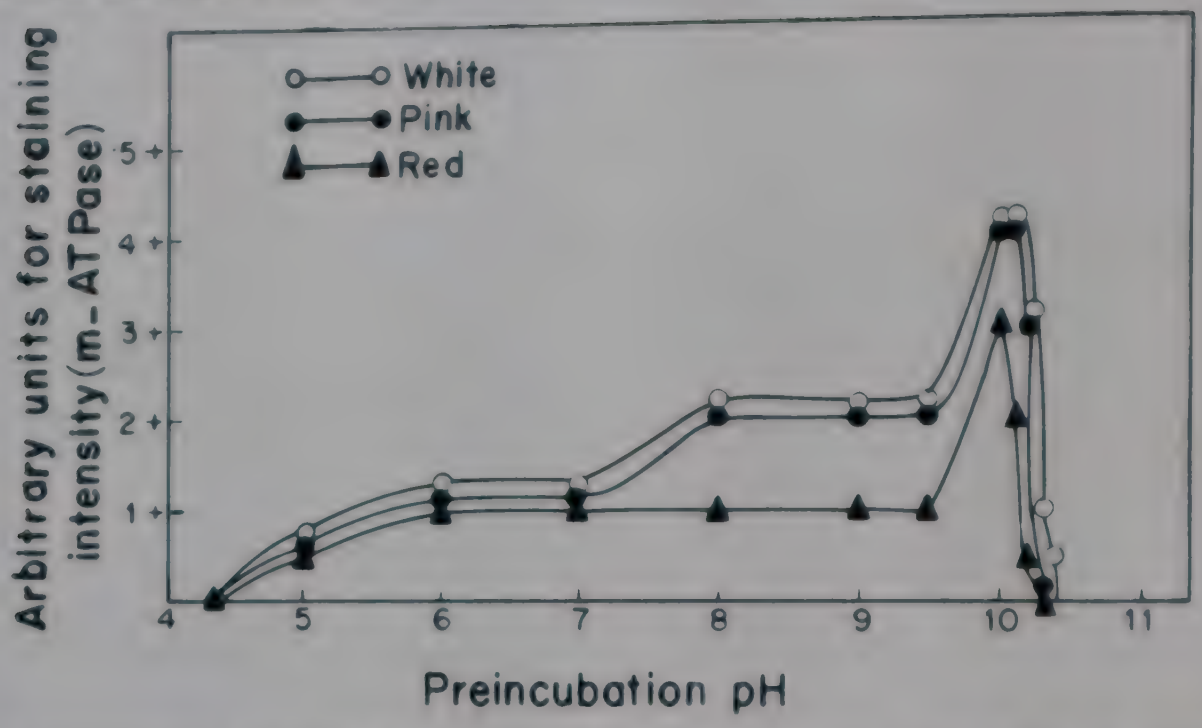


Figure 13 Histochemical *p*H-profile for the various muscles of adult *Labeo rohita* (based on observations as shown in table 1). Muscle sections preincubated at varying *p*H (4.35–10.4), PI time (5–10 sec.) and incubated routinely at *p*H 9.4 for m-ATPase

muscles are more resistant in these *p*H media (figures 13 and 14). The mean diameter of the fibre types have been presented in table 3.

Pre-Adult Muscle Fibres

Histochemically, in the 1½-month-old young fish the fibre types were found to be differentiated into red and white regions with a very narrow zone of pink fibres showing intermediate staining for SDH (figure 9). The m-ATPase reaction showed quite low intensity in the red zone whereas the white and pink fibres had shown good intensity of staining (figure 11). In the 3½-month-old fish, the red and pink fibre zones were quite well developed (figure 10) and were found to stain intensively for m-ATPase in the alkali *p*H 10.0 (5–10 sec.) as shown in the adult muscle fibres (figures 3 and 12).

Table 3 *Fibre diameter* in the various muscle fibre types in the trunk and tail myotomal regions of the freshwater teleost* Labeo rohita

Fibre types	Myotomal Region	
	Trunk	Tail
Red	15.41±1.01	19.73±0.90
White	54.28±2.06	45.55±1.82
Pink	41.00±2.34	37.35±1.59

*Values are Mean ± SE of the mean for 25–50 fibres in each case

Discussion

In *Labeo rohita* the red, pink and white muscles are segregated to such an extent that the three can be separated easily for individual type analysis.

An important point here to be observed is that the alkaline stability of pink muscle is found to be inbetween red and white which is different from what has been observed earlier in other teleosts (Johnston et al. 1974, Patterson et al. 1974, Talesara & Shashi Kiran 1982, 1983, Davison 1983). In *Labeo rohita*, pink muscle is not most resistant in the alkaline preincubation *p*H. This extreme kind of variation in the pink fibres demonstrated with the m-ATPase reaction probably points to a high polymorphism of those myosins

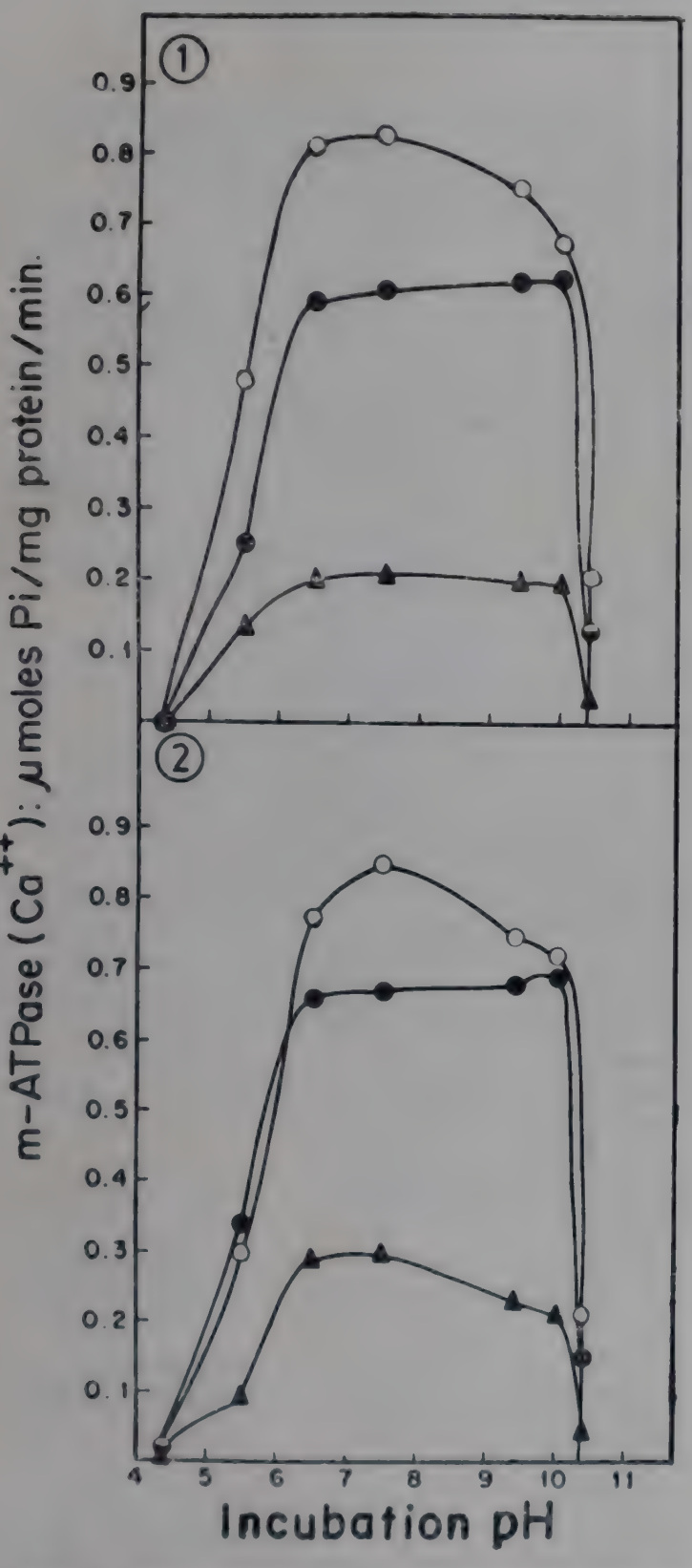


Figure 14 Biochemical *p*H—profile for m-ATPase in the various muscles of the trunk (1) and tail (2) myotomal regions of adult *Labeo rohita* (0——0—White; ●——●—Pink; ▲——▲—Red)

that react differently to alkaline treatment. These variations in the kind and amount of the pink muscle in different teleosts, leads to the assumption that they are less specialized than the white muscle and are, therefore, capable of performing a wider range of functions.

Barany (1967) has demonstrated for a wide range of vertebrate muscles that myofibrillar-ATPase activities parallel the unloaded speeds of shortening. On this basis white muscle of *Labeo rohita* is around 3-4 times as fast as red muscle. The pink muscle is found to be having m-ATPase slightly lower than white muscle, i.e. around 2-3 times higher than red muscle suggesting it to be a fast twitch muscle with an intermediate speed of shortening between the red and white muscles.

The arrangement of the fibre types in the pre-adult stages is found to be basically like the adult ones. The predominance of white muscle fibres in the early free-living stage ($1\frac{1}{2}$ month) reflects the burst type of swimming behaviour of the young ones. As the fish grows, there is a gradual increase in the size and 'red like' character of the myotomal population correlating with a gradual transition from rapid burst type activity to a steady, sustainable swimming behaviour as observed in the rearing tanks. This basically agrees with the earlier reports (Waterman 1969, Nag & Nursall 1972).

To conclude, the present investigations of the myotomal musculature of the teleost fish, *Labeo rohita*, reveal three types of muscle fibres i.e. red, white and pink. The superficial red fibres are slow twitch red-type I fibres suitable for slow, sustained swimming. The white fibres are classified as fast twitch white-type IIB, suitable for rapid burst swimming for short periods. The third fibre type, is the pink which can be fast twitch red-type IIA or C probably best suited for activity ranges otherwise too high for red muscle.

Acknowledgements

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Oxygen Uptake in Relation to Body Weight, Respiratory Surface Area and Group Size in a Freshwater Goby, *Glossogobius giuris* (Ham.)

O N SINGH and J S DATTA MUNSHI
Post-Graduate Department of Zoology, Bhagalpur University, Bhagalpur 812007

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Oxygen consumption ($\dot{V}o_2$) has been studied in relation to different body weight, respiratory surface area and group size in a fresh water goby *Glossogobius giuris* (Ham.)

The relationship between body weight and $\dot{V}o_2$ was found to be:

(*a*) $\dot{V}o_2 = 0{\cdot}321\,W^{0{\cdot}632}$ (when both adult and juvenile fishes were considered together); (*b*) $\dot{V}o_2 = 0{\cdot}320\,W^{0{\cdot}688}$ (in juveniles); and (*c*) $\dot{V}o_2 = 0{\cdot}391\,W^{0{\cdot}556}$ (in adults only)

The relationship between the total respiratory surface area (A) and oxygen consumption $\dot{V}o_2$ was found as: $\dot{V}o_2 = 0{\cdot}00045A^{1{\cdot}2358}$. The two parameters are positively correlated as indicated by high correlation coefficient (*r*).

Effect of group size or number of individuals indicated that $\dot{V}o_2$ /unit body weight (ml/g/hr) was lower in grouped fishes or fish in a larger group than when they are in isolated condition or in a smaller group. Significant but negative correlation were found between these two parameters.

Key Words: Oxygen uptake, Body weight, ***Glossogobius giuris***

Introduction

Oxygen consumption has long been considered a valid measure of metabolism. In recent years, several workers have studied the relation between oxygen consumption and body weight in fish (Parvateshwararao 1959, 1960, Hemingsen 1960; Saunders 1963, Brett 1965, Paloheimo & Dickie 1965, Kamler, 1972, Munshi & Dube 1973, Lomholt & Johansen 1976, Hughes 1977, 1978, Munshi et al. 1978, 1982 and Hakim et al. 1983). Attempts have also been made to correlate oxygen consumption with respiratory surface area (Ojha & Munshi 1975, Munshi et al. 1982 and Hakim et al. 1983).

Effect of group size on oxygen uptake in fishes has been studied by several workers (Allee 1931, Schuett 1933, Bowen 1932, Shtefanesku 1958, Malyukina et al. 1962, Grigor'eva 1966 and Itazawa et al. 1978).

In the present work the effect of body weight, respiratory surface area and group

size on oxygen consumption of a fresh water goby *Glossogobius giuris* (Ham.) has been studied.

Materials and Methods

Live specimens of *Glossogobius giuris* (Ham.) of different body weights were collected from local ponds of Bhagalpur and maintained in the glass aquarium in the Laboratory for acclimatization. They were fed with goat liver and intestine. Feeding was stopped 24 hr before the start of experiment.

A cylindrical glass respirometer of 1.5L was used to measure the oxygen consumption ($\dot{V}o_2$) rate. The flow of water from the reservoir to the respirometer was maintained according to the size of the fish to avoid suffocation and stress. Two series of water samples were taken, one from the water supplied to the respirometer and the other of the water coming out of respirometer. The oxygen content of the water samples thus collected were measured by Winkler's volumetric method (Welch 1948). The difference between the oxygen content of the incoming and outgoing water together with the water flow rate (ml/h) through the respirometer were used to calculate the oxygen consumption rate. Three to four readings were taken at hourly interval for each fish. These experiments were carried out at temperature $22 \pm 1°C$.

To study the effect of group size on the oxygen uptake, fishes of the same weight group (12.8–14.2g) were chosen. Firstly, the oxygen consumption of one fish was measured in the above manner. Then oxygen consumption of groups having 2, 3, 4, 5, 6 and 7 fishes together were measured respectively. To avoid suffocation and stress, water flow was increased with increasing number of fishes.

These experiments were carried out at temperature $25.0 \pm 0.5°C$.

Regression analysis using logarithmic transformations was done to show the relation of oxygen consumption with body weight, respiratory surface area and group size.

Results

Data on the oxygen consumption ($\dot{V}o_2$) in relation to body weight, respiratory surface area and group size have been summarized in tables 1–3. Relationship of oxygen consumption with its respective parameter has been shown in table 4 and figures 1–4.

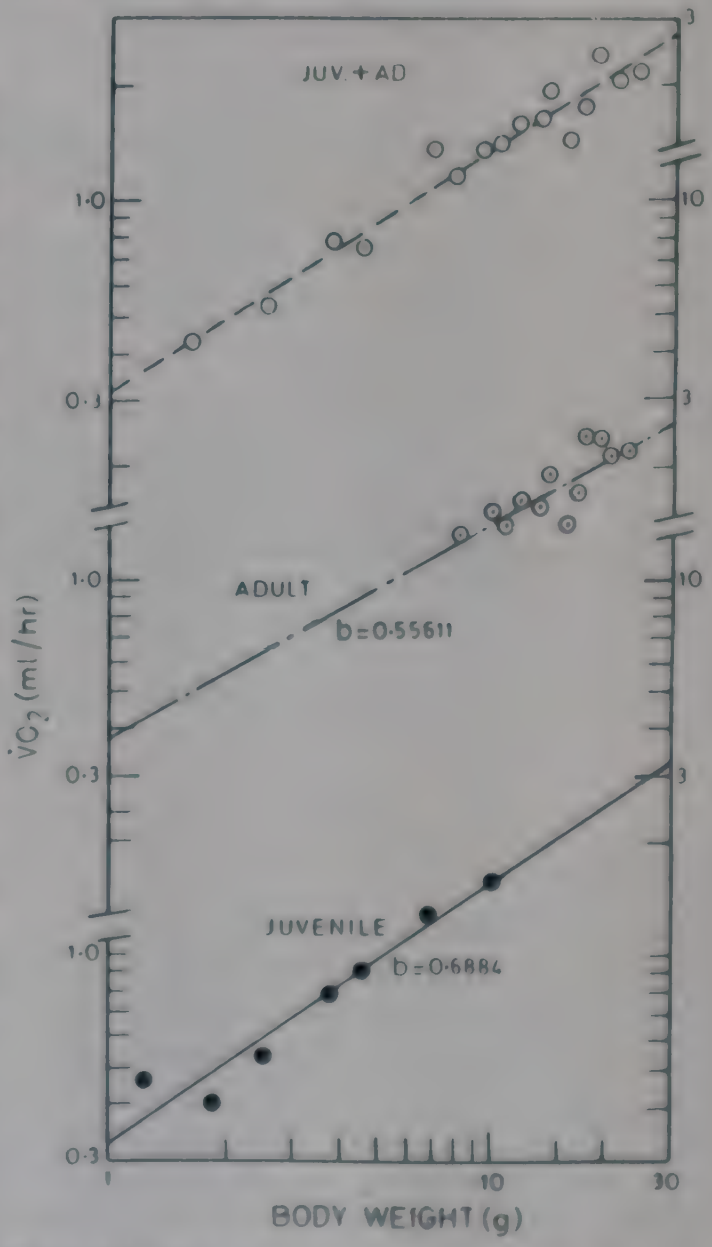

Figure 1 Log/log plots showing relationship between body weight and oxygen uptake (mlO_2/hr) of *Glossogobius giuris*

Table 1 *The rate of oxygen consumption ($\dot{V}o_2$) in ml/hr and ml/g/hr against body weight of* Glossogobius giuris *(Ham.) at* 22±1°C

Average body wt (g)	Oxygen consumption ($\dot{V}o_2$)	
	ml/hr	ml/g/hr
1·6	0·411	0·2718
2·5	0·539	0·2300
3·8	0·817	0·1970
4·6	0·749	0·1836
7·3	1·377	0·1547
8·3	1·332	0·1479
10·2	1·485	0·1369
11·0	1·366	0·1329
12·0	1·587	0·1287
13·5	1·564	0·1232
14·3	1·905	0·1206
16·5	1·423	0·1144
17·7	1·670	0·1115
18·7	2·393	0·1092
19·3	2·410	0·1079
20·4	2·167	0·1059
23·2	2·135	0·1008

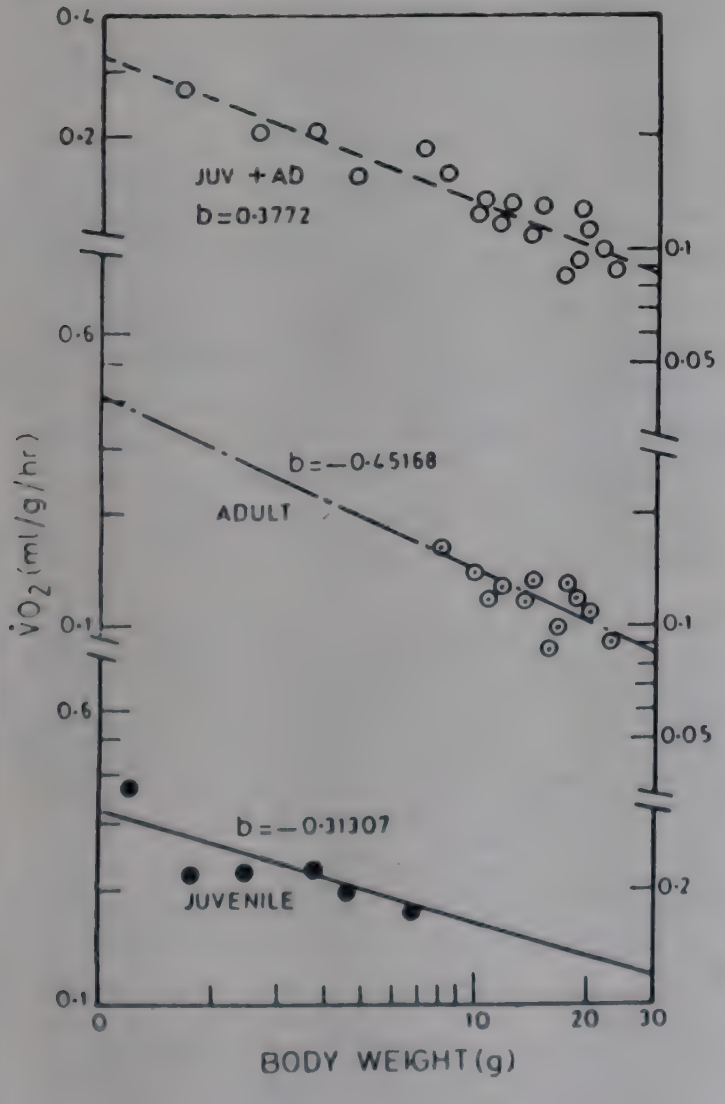

Figure 2 Log/log plots showing relationship between body weight and oxygen uptake (mlO_2/g/hr) of *Glossogobius giuris*

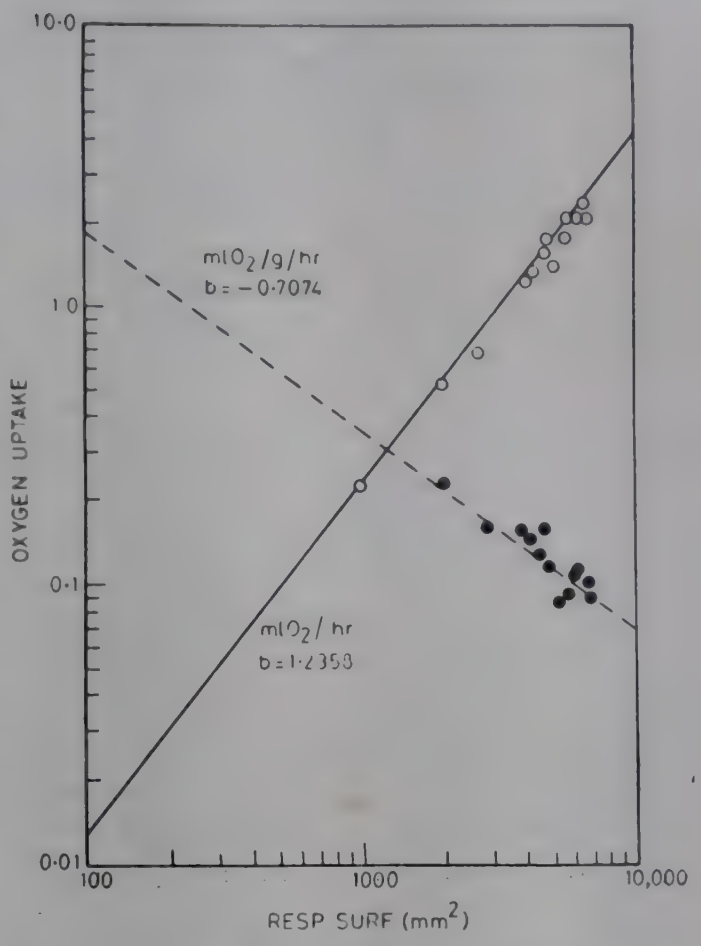

Figure 3 Log/log plots showing relationship between O_2 uptake and respiratory surface area of *Glossogobius giuris*

Oxygen Consumption in relation to Body Weight

The oxygen consumption (ml/hr) of *Glossogobius giuris* ranged from 0.411 to 2.410 within the weight range of 1.6 to 23.2g. This increase of Vo_2 was in a parabolic fashion.

The log/log plots of $\dot{V}o_2$ (ml/hr) and body weight gave a straight line and the following relationship is applicable :

$$log\ \dot{V}o_2 = log\ 0.321 + 0{\cdot}632\ log\ W$$

or $$\dot{V}o_2 = 0.321\ W^{0.632}$$

The equation indicates that $\dot{V}o_2$ increases with increasing body weight by a power of 0.632.

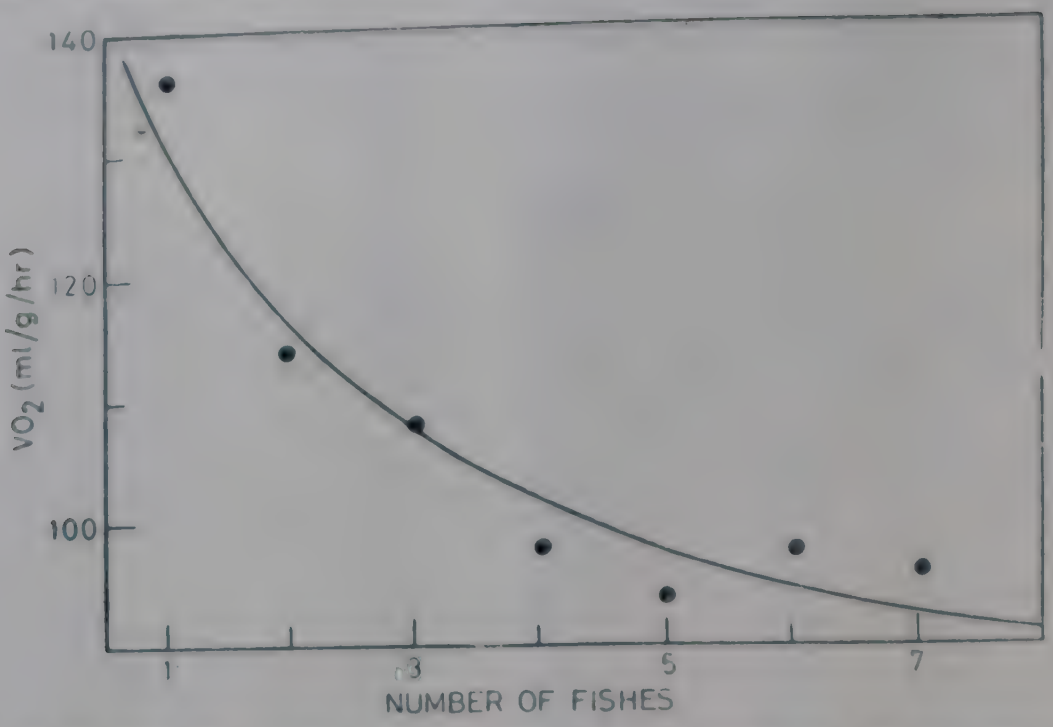

Figure 4 Relationship between group size and oxygen uptake (mlO_2/g/hr) of *Glossogobius giuris*

Table 2 *Respiratory areas (A) and O_2 consumption for different weight group of* Glossogobius giuris

Body wt (g)	Resp. surf. area (A)	Oxygen uptake ($\dot{V}o_2$)	
		(ml/hr)	ml/g/hr)
2·5	2013·65	0·539	0·216
4·6	2744·80	0·749	0·163
8·3	3760·03	1·332	0·160
10·2	4175·12	1·485	0·146
12·0	4534·44	1·587	0·132
13·5	4814·04	1·564	0·116
16·5	5330·67	1·423	0·086
17·7	5524·21	1·670	0·094
18·7	5680·62	2·393	0·128
19·0	5726·73	2·121	0·111
20·4	5934·34	2·167	0·106
23·2	6338·23	2·135	0·092

The weight specific oxygen consumption rate (ml/g hr) decreases with increasing body weight in a parabolic manner. The decrease is sharp in the younger weight groups and lesser in older ones.

The rate of oxygen uptake per unit body weight within the body weight range of 1.6 – 23.2 g was found to be 0.272 to 0.1008 ml/g/hr. When the log $\dot{V}o_2$ (ml/g/hr) were plotted against log body weight, a straight line was obtained, with a slope of –0.377. The estimated value of $\dot{V}o_2$ for 1g fish was 0.3287 ml/g/hr. The relation between the two variables can be shown by following equations:

$$log\ \dot{V}o_2 = log\ 0.3287 - 0.377\ log\ W$$

or $$\dot{V}o_2 = 0.3287\ W^{-0.377}$$

The correlation coefficient (r) for the weight related increase and decrease in oxygen uptake rate (ml/hr) and ml/g/hr) was 0.9680 ($P<0.001$) and –0.9175 ($P<0.001$).

When $\dot{V}o_2$ (ml/hr) was measured for juveniles and adult fishes separately, the regression coefficient of the log $\dot{V}o_2$/log W plots gave different values. In the case of juveniles, the slope value was greater ($b=0·688$) while in the case of adult it was less ($b=0.556$). However, in both the casse

Table 3 *Effect of group size (weight range* 12·5g–14·5g*) on oxygen consumptiono f* Glossogobius giuris

No. of Fishes	Total wt (g)	Avg wt (g)	O_2-uptake (ml/g/hr)
One	13·5	13·50	137·35
Two	26·5	13·35	114·80
Three	39·4	13·13	108·63
Four	56·7	14·17	98·32
Five	70·7	14·14	94·60
Six	82·6	13·76	98·47
Seven	90·5	12·90	97·80

Intercept '*a*', 133·037; Slope '*b*', 0·1842; Correlation coefficient '*r*', –0·95603

Table 4 *Regression coefficient (b), Intercept (a) and correlation coefficient (r) to show the relationship of oxygen consumption (Vo_2) (ml/hr and ml/g/hr) to body weight and respiratory surface area*

Parameters	Regression coefficient (*b*)		Intercept (*a*)		Correlation coefficient (*r*)	
	(ml/hr)	(ml/g/hr)	(ml/hr)	(ml/g/hr)	(ml/hr)	(ml/g/hr)
Oxygen Consumption in relation to Body Weight						
1. Juvenile & Adult	0·6318	—0·3772	0·3124	0·3287	0·9680(*P* 0·001)	—0·9175(*P* 0·001)
2. Juvenile	0·6884	—0·3131	0·3201	0·3205	0·9491(*P* 0·001)	—0·8023(*P* 0·001)
3. Adult	0·5561	—0·4517	0·3909	0·4003	0·8029(*P* 0·001)	—0·7345(*P* 0·001)
Oxygen Consumption in relation to Respiratory Surface Area						
1. Juvenile & Adult	1·2358	—0·7074	0·000045	47·8507	0·9628(*P* 0·001)	—0·8953(*P* 0·001)

the correlation coefficient value was high and significant (juvenile, $r=0.94910$; $P<0.001$; adult, $r=0.80291$; $P<0.001$). The relationships are summarised in the following equations :

In juveniles $\dot{V}o_2=0.3201\ W^{0.688}$
In adults $\dot{V}o_2=0.3909\ W^{0.556}$

Oxygen Consumption in relation to Respiratory Surface Area

The rate of oxygen consumption ranged from 0.539 to 2.393 ml/hr for fish having respiratory surface areas ranging from 2013.65 to 6338.23 mm² (table 3). The log/log plots of respiratory surface area of gill and the corresponding oxygen consumption gave straight line with slope of 1.2358 and estimated intercept 0.000045. Thus the relation between the above two variables can be shown in logarithmic form as

$$log\ \dot{V}o_2=log\ 0.000045+1.2358\ log\ A$$
or $\dot{V}o_2=0.000045\ A^{1.2358}$

There is high and significant correlation between total respiratory surface area and $\dot{V}o_2$ ($r=0.9628$; $P<0.001$).

The weight specific fall in $\dot{V}o_2$ (ml/g/hr) is comparatively high. For unit increase in respiratory surface area, the fall would be by a power of −0.7074. The weight specific fall in $\dot{V}o_2$ appears to be highly correlated with the respiratory surface area ($r=0.89528$; $P<0.001$).

Oxygen Consumption in relation to Group Size

It was observed that $\dot{V}o_2$ (ml/g/hr) was lesser in grouped fishes or fish in a larger group than that in an isolated fish or fish in a smaller group. This fall is sharp till the number of fishes in group is four, after that oxygen consumption (ml/g/hr) becomes more or less constant.

There is a high but negative correlation between oxygen consumption per unit body weight and the group size as indicated by the high and negative value for correlation coefficient ($r=-0.972$; $P<0.001$). The slope of regression line was −0.18421.

Discussion

The oxygen consumption within a single species depends on body size (Rübner 1883). He found metabolic rate of dogs to be proportional to $W^{0.67}$. Metabolism is correlated inversely to body weight. It has been suggested by Brody (1945) and Zeuthen (1953) that a value of 0.67 is approximate for homeotherms. Hemingsen (1960) found 0.75 ± 0.015 (SE of slope) was not unusual for poikilotherms. Prosser and Brown (1961) made a broad generalization that values may range from 0.67 to 1.0. The lower value is an expression of the surface area to volume rate which suggests that surface area varies to the 2/3 power of body size. Almost all values have been found in this range or below it. A value less than 1.0

would indicate weight specific metabolic rate decreasing with increasing size. Zeuthen (1953) however asserted that values greater than 1.0 are exceptions to the rule.

In *Glossogobius giuris* the exponent value for $\dot{V}o_2$ in relation to body weight was found to be 0.632. This value is lower than the exponent values reported for a number of purely aquatic fishes (table 5). This is an indication of a lower rate of metabolic increase with unit increase in body weight in *G. giuris*. However, the values reported for this goby is higher than the values reported for air-breathing fishes like *Channa punctata* (0.620), *Anabas testudineus* (0.550) and *Colisa fasciatus* (0.595), under the same experimental condition.

When exponent values of juveniles and adults were considered separately, two components of the regression line with slope values 0.556 in the case of adults and 0.688 in juveniles were found. The intraspecific variations in the exponent values may be due to different growth pattern and physiology of juveniles and adults. Kamler (1972) has reported intraspecific variations in the exponent values of *Cyprinus carpio*, higher exponent value (0.98) for smaller fishes less than 1000 mg and lower values (0.80) for higher weight groups of fishes. Munshi et al. (1978) have also reported different exponent values for juvenile (b=1.084) and adult fish (b=0.986) in their studies of *Heteropneustes fossilis*.

Table 5 *Exponent values reported for oxygen uptake $\dot{V}o_2$ (ml/hr) and body weight in some Indian air-breathing and aquatic fishes.*

Fish Species	Slope (b)	References
Anabas testudineus	0·530	Munshi & Dube 1973
Clarias batrachus	0·841	Munshi et al. 1976
Colisa fasciatus	0·595	Ojha et al. 1977
Channa punctata	0·620	Hakim et al. 1983
Boleophthalmus boddaerti	0·791	Biswas et al. 1979
Salvelinus fontinalis	0·850	Job 1955
Acipenser stellatus	0·735	Jager & Dekkers 1975
Anguilla anguilla	0·794	–do–
Mystus cavasius	0·662	Ojha & Singh 1981
Macrognathus aculeatum	0·709	Ojha & Munshi 1975
Cirrhinus mrigala	0·811	Roy 1983
Glossogobius giuris	0·632	Present author

The slope of regression line for weight specific decrease in Vo^2 was —0.377, when both adults and juveniles were considered together. But when adults and juveniles were taken separately this value was found to be —0.313 and —0.4517 for adults and juveniles respectively indicating fall in $\dot{V}o_2$ (ml/g/hr) with unit increase in body weight. For thermodynamic reasons the metabolic rate can never increase, but only decrease with evolution of body size in organisms (Prigogine & Wiame 1946). The decreasing trend in the metabolism may be due to the fact that the sum of enzymes, metabolites and perhaps inhibitors which govern metabolic activity of organism, changes with age (Zeuthen 1953). The other factor responsible for the decrease of metabolic rate in higher weight group of fishes is the sluggish behaviour of older fishes in comparison to younger ones.

In *Glossogobius giuris*, the total respiratory area increase by a power of 0.508 (Singh 1982). It may be presumed theoretically that the oxygen consumption in relation to body weight should increase by a power of 0.508 or nearer to this value. But it is obvious from the present investigations that the $\dot{V}o_2$ increases by a power of 0.632. Muir and Hughes (1969) found for tunny that relationship of area to weight is similar to that between oxygen consumption rate (ml/hr) and body weight. Ultsch (1974) also reported similar situation in three species of sirenidae. However, in subsequent studies on other fishes it has been found

that the slope of the regression lines for total gill area is not always the same as that of the slope of the $\dot{V}o_2$/body weight relationship.

The oxygen consumption rate of an animal depends considerably on the dimensions of the respiratory surface area. Hemingsen (1960) also argued the importance of respiratory surface area while considering oxygen consumption rate. In the present fresh water goby with unit increase in respiratory surface area the $\dot{V}o_2$ (ml/hr) increases by a power of 1.2358. The weight specific fall in $\dot{V}o_2$ (ml/g/hr) in relation to respiratory surface area is comparatively high ($b = -0.7074$). However, the surface area available for obtaining O_2 is not the only factor that will determine the ability to take up O_2. The blood-water barrier is the other important parameter that influences the O_2 uptake.

The oxygen consumed by this fresh water fish in groups of more than onep resents an interesting picture. From the results obtained it is clear that *G. giuris* reduce their oxygen uptake while in groups, whereas an isolated fish or fish in smaller group exhibit a higher rate of oxygen consumption. Similar observations have been made by Allee (1931), Malyukina et al. (1962), Grigor'eva (1966), Shtefanesku (1958). Allee (1931) in summarizing research to that date assumed that 'group effect' lowers down the oxygen consumption of fishes. Malyukina et al. (1962), Grigor'eva (1966) and Shtefanesku (1958) all have reported an inverse relationship of group size to oxygen consumption. Itazawa et al. (1978) while studying the group effect on the O_2 uptake of the rainbow trout and the medaka found similar results and concluded that fish in a larger group feel at ease and reduce their oxygen uptake. However Bowen (1932) found no such significant disismilarity between grouped and isolated catfish, *Ameriurus melas.*

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Influence of Upper and Lower Temperature Extremes on Swimming Performance of Freshwater Mullet, *Rhinomugil corsula* (Hamilton)

N SUKUMARAN

Fisheries College, Tamil Nadu Agricultural University, Tuticorin 628008

(Received 7 August 1984; after revision 22 January 1985)

Freshwater mullet, *Rhinomugil corsula* acclimated to 30°C in freshwater and forced to swim at current velocities of 38, 62 and 77 cm S^{-1} in Blazka's swimming chamber failed to swim at 35·2, 34·6 and 34·2°C respectively, when the temperature of ambient water was gradually increased from the acclimation temperature. The corresponding lower critical temperatures experimented were 26·2, 27·5 and 28·1°C.

The pattern of swimming failure at critical temperature was similar to that at critical ambient concentrations of oxygen and other pollutants. Since such failures of swimming do not incapacitate the fish from further performance and critical temperatures can be considered as points in a temperature gradient in running water, it appears that these temperatures are indicative of a thermal avoidance in running water.

Key Words: *Rhinomugil corsula*, **Temperature extremes, Swimming performance**

Introduction

With the ever-increasing need for power, thermal power generation units are likely to be increased. A good amount of water from natural systems, rivers, lakes or sea has to be pumped into the said units for cooling and the used water is discharged back into the same area and this may considerably increase the temperature of the medium. While the lethal response is recognised as the most important of the critical thermal responses, the sublethal responses are no less significant. The immediate responses of an organism to enhanced temperature are shock reactions, resulting often in death, avoidance and preference responses. The latter two behavioural responses are significant, for these would explain the occurrence of motile fish in a particular area.

Swimming inhibition or failure to swim in a steadily swimming fish at threshold levels of ambient oxygen and temperature has already been described (Kutty 1968, Kutty & Saunders 1973, Kutty & Sukumaran 1975). However, it is imperative to describe behavioural responses of fish, as the available literature mostly deals with lethal effects only (Nakatani 1971, Coutant 1970, Moss 1970). In addition, fatigue collapse of actively swimming fish at various

temperatures has also been investigated (Brett 1967). The critical temperatures at which fish swimming in a current of water fail to swim (the point at which swimming inhibition takes place) can be investigated experimentally by gradually changing the ambient temperature of a water current (Kutty & Sukumaran 1975). In the present study, such critical temperatures of swimming failure in freshwater mullet, *Rhinomugil corsula*, swimming steadily in induced water current have been investigated. Since such failures do not incapacitate the fish from further performance and since these critical temperatures can be considered as points in a temperature gradient in running water, it is possible that these temperatures are indicative of some level of temperature avoidance.

Materials and Methods

Freshwater mullets, *Rhinomugil corsula* (mean size 17.2 cm) were collected from the Vaigai reservoir and were maintained in acclimation tanks at 30 $\pm$ 0.5°C for two weeks prior to experimentation. During acclimation, they were fed daily with a formulated diet of ground fish muscle, goat liver and wheat hearts in a ratio of 2 : 1 (Narayanan 1974). The experimental fish were then starved for 24 hr prior to subjecting them for tests. The oxygen content in the acclimation and the test tanks were kept at 80–100% air saturation.

A Blazka respirometer (Blazka et al. 1960), a tunnel-type double cylinder activity apparatus, was used for exercising the fish. Desired water current velocities were created in the inner cylinder (Swimming chamber) by the rotation of a motorized propeller controlled by a voltage regulator as described by Kutty and Sukumaran (1975). The experimental fish was introduced into the swimming chamber which was closed at the hind end by a grid attached to a door. An area of 4 cm at the rear end of the swimming tunnel, in front of grid was marked out as "reverse" or "retreat" areas. Water was flushed through or allowed to stagnate in the apparatus, while the water velocities were maintained at desired levels. The present unit was adopted for swimming behaviour studies so that a photocell relay attached near the door and of the swimming chamber could monitor the position of the test fish. The fish normally oriented to the water current and remained in the front portion of the chamber, provided by black plastic cover. An increase in the quantum of stress factor in the ambient water towards the critical level induced the fish to lag or retreat or reverse from the curtained front area and come to the hind end of the swimming chamber (Kutty & Sukumaran 1975). The photocell relay was activated in this process and a record of the event, called as 'reverse' or 'retreat', was made, when the water quality with reference to the specific stress factor, changed closer towards the critical level, the fish made frequent reverses and usually the fish tail touched the grid. This 'grid touch' and earlier 'reverse' were taken as indices of swimming failure (Kutty & Saunders 1973, Kutty & Sukumaran 1975).

The test fish acclimated at 30 $\pm$ 0.5°C, was left in the activity apparatus for a minimum period of 4 hr undisturbed, and the tap water was simultaneously flushed through the unit. The fish was subsequently forced to swim at the selected speeds (38, 62 and 77 cm sec^{-1}), for a period of 30–60 min. In the case of upper critical temperatures, heated water was allowed to flush through the swimming chamber, causing the ambient temperature to rise gradually for the next half an hour. Swimming failure at critical temperatures was studied following the method of Kutty and Sukumaran (1975). The frequency of reverses and grid touches increased as the ambient temperature increased. If the

temperature had been further raised the fish would have collapsed since the lethal temperature was 36.2°C. Hence the incipient tests were not continued up to that point. The average of the levels of the stress factor concerned for the first 2 grid touches was taken as the critical level.

The fish was observed for some more time to confirm the pattern of its swimming failure and then the ambient temperature was brought down by flushing the swimming chamber with acclimation water. The ambient temperature was again increased to obtain a second value of critical temperature and flushed again with acclimation water. So three consecutive values of critical temperatures for single swimming speed were obtained for each experiment. After each failure, when the temperature was reduced, the fish swam up again and remained in the front portion of the swimming tunnel.

Tests for lower temperatures were conducted as for the upper temperatures, except that the temperature was brought down by flushing the swimming chamber with cold water. Tests were repeated with 4 individual fishes in each swimming speed and the average values were taken.

Results and Discussion

It is observed that the fish failed to swim, as judged from the increased frequency of "reverses" and "grid touches" when the critical temperature was reached (figure 1). In all cases, the fish swam up again when the ambient temperature was brought to the tolerance range. Data relating to three separate swimming speeds for lower and upper temperature were plotted and it is obvious that with the increase in swimming speed, the upper critical temperature decreases whereas, the lower critical temperature increases with the increase in the intensity of exercise (figure 2). That is, when the stress factor (temperature) increased, the sustained swimming showed a decrease.

The decrease in the upper critical temperature and the increase of lower critical temperature, narrowing the zone within which the fish is active, with increase in the intensity of exercise (figure 2), suggest an interaction of the two stress factors as observed by Kutty and Sukumaran (1975). The trends of "reverse" and "grid touches" during swimming failure (figure 1) were similar to that of *Tilapia mossambica* at critical temperatures (Kutty & Sukumaran 1975), critical oxygen concentrations (Kutty & Saunders 1971) and critical concentrations of biocides (Sukumaran et al. 1981). This suggests that there is a common mechanism in operation. The swimming failure observed was not the same as fatigue collapse, for the fish would swim again as soon as the apparatus was flushed with acclimation water. (Kutty & Saunders 1973, Kutty & Sukumaran 1975, Sukumaran et al. 1981). Further, there was no loss of co-ordination of locomotor activity, except the leg in swimming, apparently caused by the reduction in tail beat frequency (Kutty & Saunders 1973, Bainbridge 1958).

Even though the size of the swimming tunnel was sufficient to make a 'u' turn, none of the fish tested attempted to swim downstream, possibly because of the strong rheotactic sense and the fish could avoid the upstream unfavourable condition quite efficiently by reducing its locomotor activity and losing its pace in the water current. It would be pushed down to a safe position in a gradient condition of stress factor in running water.

As soon as the thermal stress is released by bringing the ambient temperature back to the acclimation level, the fish swim regularly again (figure 1). It is assumed that the swimming failure is a behavioural response and not a fatigue response (Kutty & Sukumaran 1975). Since the swimming failure does not incapacitate the fish from further swimming and the critical temperatures

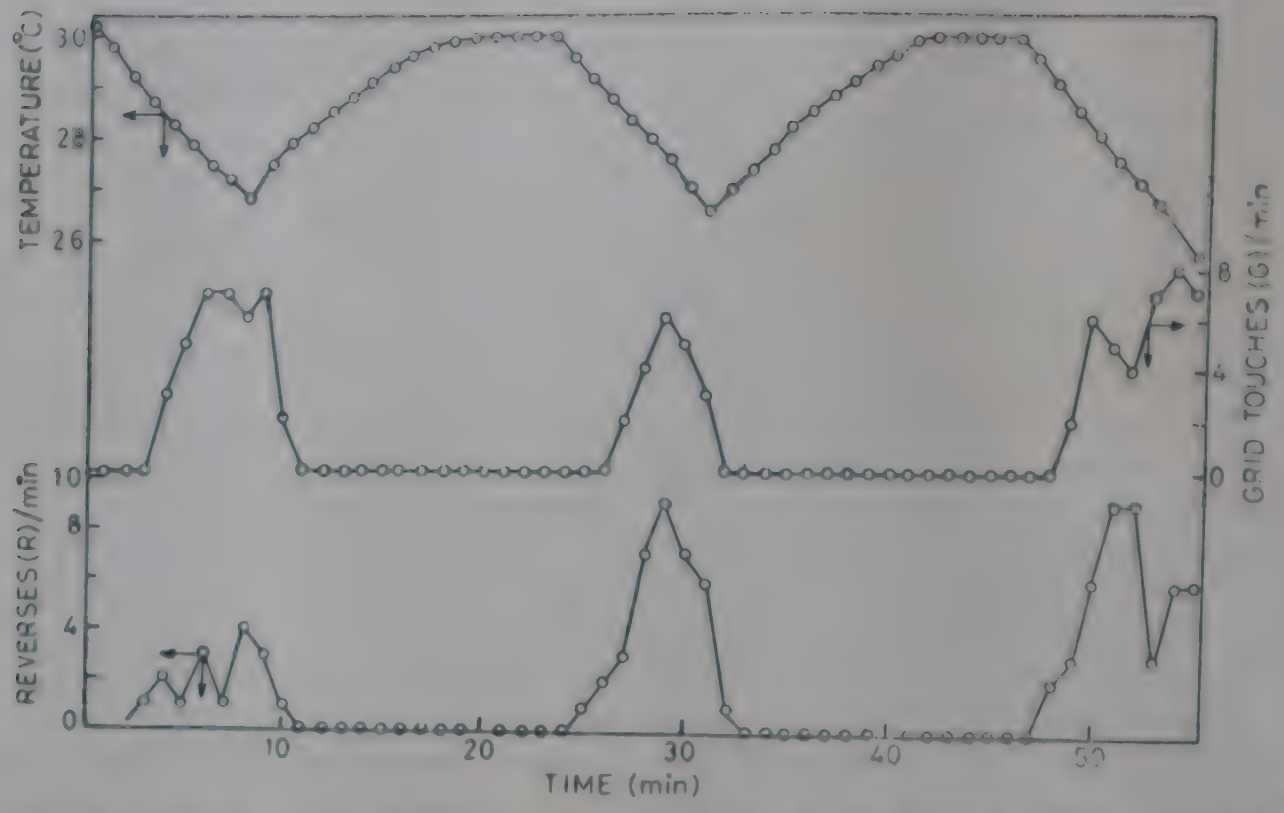

Figure 1 Behavioural responses of *Rhinomugil corsula* (17·2 cm) forced to swim in a current speed of 66 cm/sec at temperatures below the acclimation level (30°C). The figure denotes a typical experiment at particular swimming speed. When the temperature was brought down each time the fish failed to swim, as indicated by number of 'reverses' and 'grid touches', presented in lower and middle ponds respectively

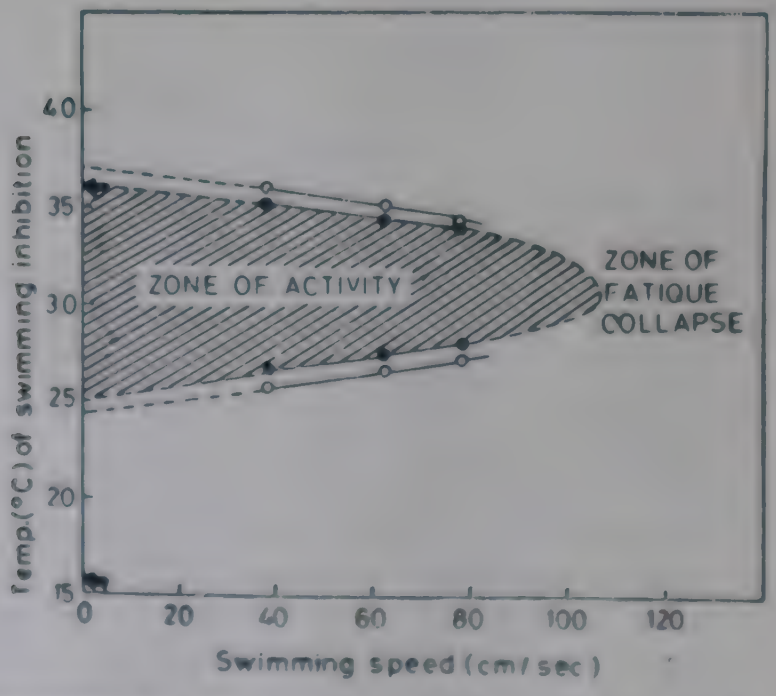

Figure 2 Relation of temperature to swimming activity of *R. corsula* acclimated to 30 C. The arrows on Y-axis indicate upper and lower incipient lethal temperatures for the same fish. The lines drawn through circles denote response of fish within a half hour to the test condition and those through the triangles denote response of fish conditioned for 1–2 hr to the experimental schedule

can be taken as points in a temperature gradient in running water, it is possible that these critical temperatures are avoidance temperatures under the specific conditions.

It is also suggested that a positive rheotactic response might induce the fish to enter heated running water, which would otherwise be avoided. Moss (1970) observed in young American shad (*Alosa sapid sima*) that "any stimulus like increased temperature has to be large enough to overcome the positive rheotaxis before it could be observed as a measurable influence". From this point of view, the critical temperatures of swimming inhibition can be taken as the thermal level at which the thermal response begins dominating the rheotactic response. Thus the fish could avoid the higher temperatures over critical level in running water and hence it is termed as 'rheotactic avoidance' (Kutty & Sukumaran 1975).

Since the critical temperatures of swimming inhibition are the possible points in a thermal gradient in running water systems, different points of avoidance for this freshwater mullet are shown in figure 3. It is assumed that ambient temperature changes @ $1°C.10m^{-1}$ from the source of heated effluent, starting with an initial temperature of 42°C. It is worthmentioning that *R. corsula* will invariably try to confine itself to lower temperatures rather than the incipient lethal temperature (figure 2). For example, in a water current of 50 cm sec^{-1} speed the mullet would fail to swim at 35.2°C avoiding higher temperatures. Further, it is reckoned that at a gradient of $1°C.10m^{-1}$, the upper thermal margins of distribution could be about 50m apart (figure 3). That is, a mullet swimming in a water current of 50 cm. sec^{-1} speed would be stopped 65m apart from the discharge of the heated effluent with a temperature of 42°C. Though further studies are required to corroborate the above findings, it is hoped that the present investigation will be of immense use in studies relating to the distribution of freshwater mullet particularly in area of heated effluent discharge.

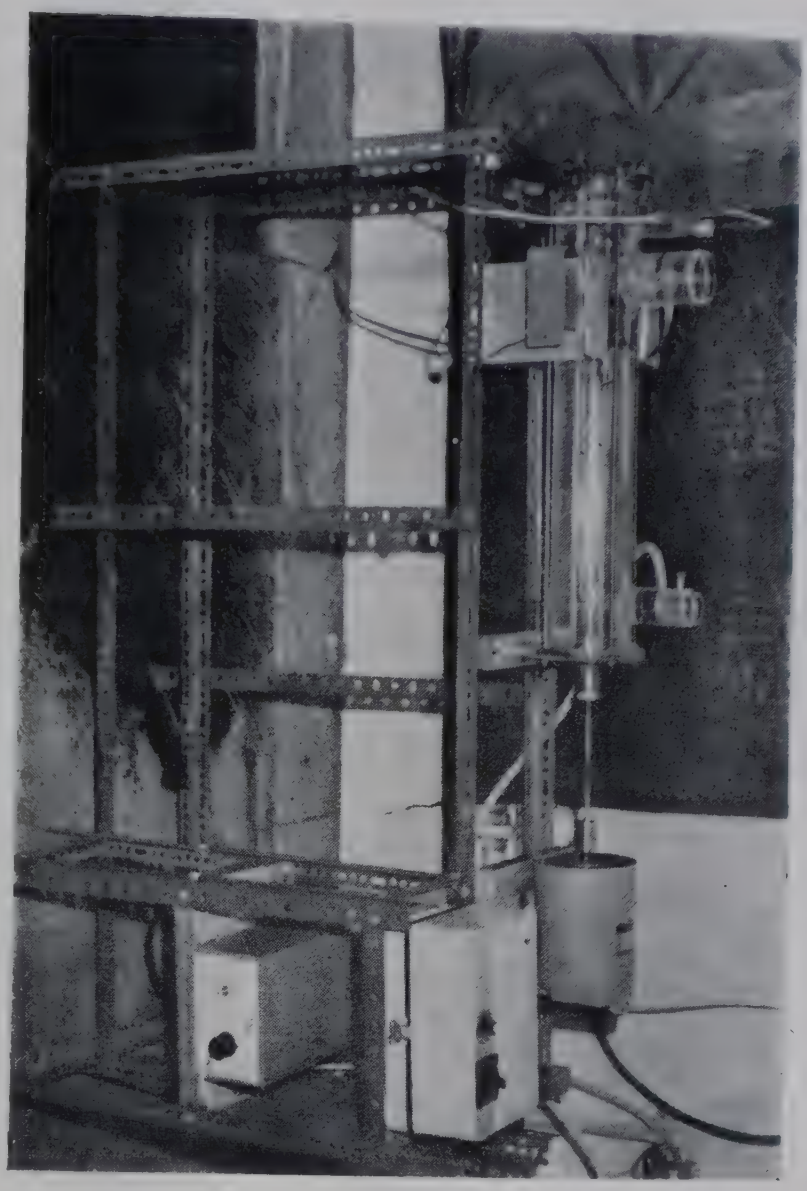

Figure 3 The thermal inhibition curve of *R. corsula* acclimated to 30°C (for details see text)

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The Effect of Water Hardness on the Respiration of the Freshwater Prosobranch Snail *Bellamya bengalensis* (Lamarck)

M BALAPARAMESWARA RAO and D SATYA REDDY
Department of Zoology, Nagarjuna University, Nagarjunanagar 522510

(Received 15 September 1984)

The metabolic rate of a tropical freshwater snail *Bellamya bengalensis* (Lamarck) in three categories of water viz., soft, medium and hard waters, has been investigated. The results of ANCOVA, comparing the slopes and intercepts of the regression lines for the sex-water category combinations, have revealed that the oxygen-consumption of the snail is related to water hardness. The metabolic rate of both the sexes was low in soft and high in hard waters. While there was no difference in the metabolic rate between male and female snails in soft and medium waters, the rate was higher in females than in males in hard water.

Key Words: Water hardness, Respiration, Snail

Introduction

Rates of respiration in the gastropod molluscs depend on many extrinsic factors and one such factor is the hardness of water. Studies on the oxygen consumption of aquatic gastropods in relation to hardness of water are rather scanty. Harrison (1968) studied the effects of calcium bicarbonate concentration on the oxygen consumption of the freshwater snail *Biomphalaria pfeifferi*. More recently Dussart and Kay (1980) demonstrated differences in the respiratory rates of several populations of *Lymnaea peregra* from soft, medium and hard waters.

Hardness of water is mainly due to the presence of calcium and magnesium salts, usually the bicarbonate or sulphate or both. The calcium cations are known to be involved in a variety of biological functions related to ionic and osmotic regulation, shell deposition and the maintenance of a background level of calcium in the viscera of molluscs (Greenaway 1971a, 1971b, Haley & Gibson 1971, Thomas & Lough 1974, Thomas et al. 1974, Dussart & Kay 1980). It is, therefore, expected that the relative hardness of water can influence the metabolic rate of shelled molluscs. The present investigation was undertaken with an aim to examine the respiratory ability of an Indian gastropod, *Bellamya bengalensis* (Lamarck) in soft, medium and hard waters.

Materials and Methods

Specimens of *B. bengalensis* were collected from a lentic habitat (Vengalayapalam pond) which is about 20 km from the university campus (16°24′N : 80°33′E). After removing the encrustations on the shells, the snails were acclimatized in the laboratory in tap-water (non-chlorinated) of medium hardness at temperature 27°±1°C, *p*H 8.8 ± 0.2 and oxygen tension ≃6.5 mg/l for 96 hr.

To classify waters into soft, medium and hard categories, the scheme outlined by Dussart and Kay (1980) was followed. The waters were assigned to one of the three categories on the basis of calcium and bicarbonate alkalinity as follows:

Category	Calcium ($mg.l^{-1}$)	Bicarbonate alkalinity ($mg.l^{-1} CaCO_3$)
Soft	0–9	0–29
Medium	10–40	30–80
Hard	>41	>81

Respiratory measurements were made by the method of Ganapati and Prasada Rao (1960) and the dissolved oxygen was estimated by Winkler's method (Golterman 1970).

Metabolic rates of males and females of *B. bengalensis* were determined separately in naturally occurring soft, medium and hard waters (*vide* table 1 for n). Experiments were performed on individual snails. Transfer from acclimation to experimental medium was abrupt. Experiments were run for a period of 2 hr and the time of the experiment (11.00 to 13.00 hr) was kept constant to avoid the effect of time of day on respiration of the snail. The temperature, *p*H and oxygen tension were the same in both acclimation and experimental conditions. After the experimentation the snails were shelled and the wet weight of the soft parts was determined to the nearest 0.1 mg. The allometric equation

$$\frac{Y}{X} = a\, X^{(b\text{-}1)}$$ (Newell 1970) (where $\frac{Y}{X}$ is the metabolic rate (MR) and X is the weight) is used to express the results.

The regression lines were fitted separately to establish the relationship of metabolic rate and weight in males and females in soft, medium and hard waters. The slopes and elevations (intercepts) of the regression lines for sex — water quality combinations were tested by ANCOVA (Snedecor & Cochran 1967).

Table 1 *Equations of the regression lines : log MR = log a + (b — 1) log W, relating weight (W) in grams to metabolic rate (MR) of* Bellamya bengalensis *in water of different hardness*

Category	Regression equations	
	Male	Female
Soft	log MR = —0·0413 — 0·4436 log W	log MR = —0·0293 — 0·4007 log W
Medium	log MR = 0·0224 — 0·4142 log W	log MR = 0·0639 — 0·3717 log W
Hard	log MR = 0·0886 — 0·3521 log W	log MR = 0·1261 — 0·5356 log W

n = 10 for each sex except in the water of medium hardness, where 40 males and 60 females are used

Results

The chemical composition of the three categories of water used in the present study was as follows:

Category	Calcium ($mg.l^{-1}$)	Magnesium ($mg.l^{-1}$)	Bicarbonate alkalinity ($mg.l^{-1}$ $CaCO_3$)
Soft	2	2	2·5
Medium	16	8	30·1
Hard	248	152	100·0

The relationship of the metabolic rate and weight of males and females in soft, medium and hard waters is shown in figures 1 and 2, respectively. The relationship of the body weight and metabolic rate of both the sexes is negatively linear when plotted logarithmically (the regression equations are given in table 1). The wet weight of the soft parts and the metabolic rate ranged from 0.33 to 1.24 g and 0.63 to 1.94 mg O_2. g^{-1} hr^{-1} in males and 0.52 to 1.88 g and 0.58 to 2.00 mg O_2. g^{-1} hr^{-1} in females, respectively.

The results of ANCOVA, comparing the slopes and elevations of the regression lines, are presented in table 2 for males and females, and in tables 3 and 4 among the three categories of water for males and females, respectively. The results presented in table 2 reveal no significant difference in both the slopes and elevations of the regerssion lines for males and females in soft, and medium waters ($P > 0.05$). In hard water, however, the difference between the elevations is significant ($F_{1,17} = 11.0769$: $P < 0.05$). It thus appears that the metabolic rate of males and females is not significantly different in soft and medium waters, but in hard water it is significant; the metabolic rate is higher in females than in males (figures 1 and 2).

The results presented in tables 3 and 4 suggest common slopes and elevations amongst soft and medium, medium and hard

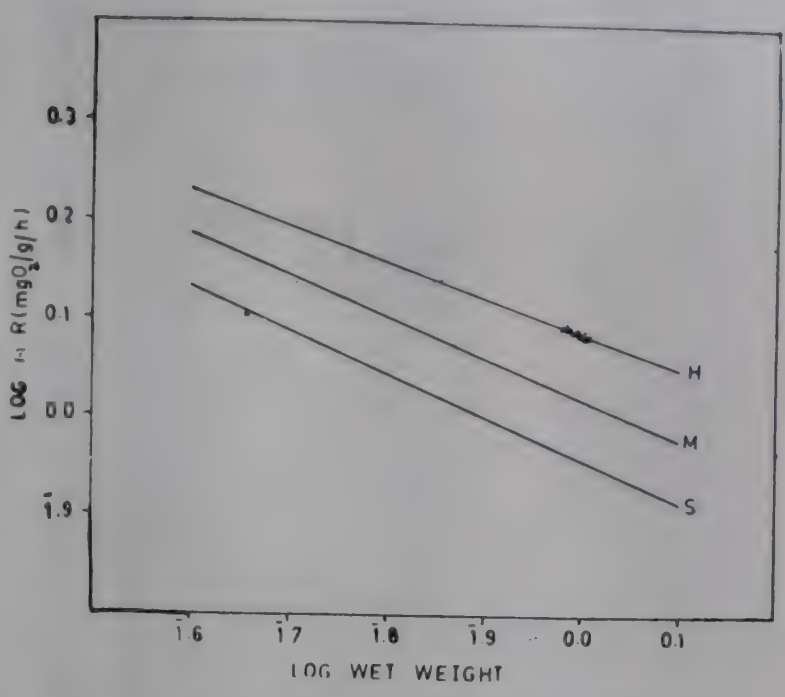

Figure 1 The relationship of log metabolic rate (MR) and log wet weight of soft tissues in males in soft (S), medium (M) and hard (H) waters

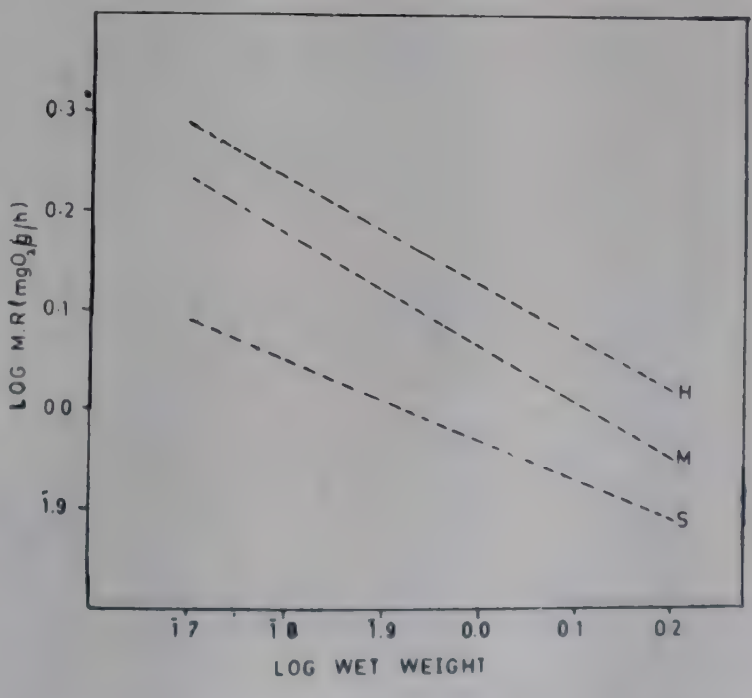

Figure 2 The relationship of log metabolic rate (MR) and log wet weight of soft tissues in females in soft (S), medium (M) and hard (H) waters

Table 2 *Results of analysis of covariance : comparison of the slopes (b) and elevations (a) of the regression lines of males and females of* Bellamya bengalensis *in waters of different hardness*

Category	Variable (Sex)	d.f.	Sxx	Sxy	Syy	(b-1)	Variation from Regression		Variance	Ratio (F)	Result
							SS	MS			
Soft	Male	9	0·0862	—0·0383	0·0291	—0·4432	0·0122	0·0015	F_{slopes}	0·0909	NS*
	Female	9	0·1339	—0·0537	0·0268	—0·4010	0·0053	0·0007	$F_{elevations}$	0·5000	NS*
Medium	Male	39	0·7950	—0·3294	0·5307	—0·4143	0·3939	0·0082	F_{slopes}	0·9208	NS**
	Female	59	0·7081	—0·4048	0·8074	—0·5717	0·5760	0·0099	$F_{elevations}$	2·7030	NS**
Hard	Male	9	0·1997	—0·0703	0·0368	—0·3520	0·0121	0·0015	F_{slopes}	3·0833	NS*
	Female	9	0·2546	—0·1363	0·0794	—0·5353	0·0064	0·0008	$F_{elevations}$	11·0769	HS*

NS, Not Significant; *HS*, Highly Significant; **d.f.*, F_{slopes} : 1, 16 and $F_{elevations}$: 1, 17; ***d.f.*, F_{slopes} : 1, 96 and $F_{elevations}$: 1, 97

Table 3 *Results of analysis of covariance : comparison of the slopes (b) and elevations (a) of the regression lines of males of* Bellamya bengalensis *between different types of water*

Variable (Category)	d.f.	Sxx	Sxy	Syy	(b-1)	Variation from Regression		Level of Comparison	Variance	Ratio (F)	Result
						SS	MS				
Soft	9	0·0862	—0·0383	0·0291	—0·4432	0·0122	0·0015				
									F_{slopes}	0·0114	NS**
								Soft and Medium			
									$F_{elevations}$	3·2674	NS**
Medium	39	0·7950	—0·3924	0·5307	—0·4143	0·3939	0·0082				
									F_{slopes}	0·0682	NS**
								Medium and Hard			
									$F_{elevations}$	3·1149	NS**
Hard	9	0·1997	—0·0703	0·0368	—0·3520	0·0121	0·0015				
									F_{slopes}	0·2687	NS*
								Hard and Soft			
									$F_{elevations}$	48·2000	HS*

NS, Not Significant; *HS*, Highly Significant; * *d.*, F_{slopes} : 1, 16 and $F_{elevations}$: 1, 17; ** *d.f.*, F_{slopes} : 1, 46 and $F_{elevations}$: 1, 47

Table 4 *Results of analysis of covariance : comparison of the slopes (b) and elevations (a) of the regression lines of females of* Bellamya bengalensis *between different types of water*

Variable (Category)	d.f.	Sxx	Sxy	Sy	(b-1)	Variation from regression		Level of comparison	Variance	Ratio (F)	Result
						SS	MS				
Soft	9	0·1339	—0·0537	0·0268	—0·4010	0·0053	0·0007				
								Soft and Medium	F_{slopes}	0·3356	NS**
									$F_{elevations}$	7·4110	S**
Medium	59	0·7081	—0·4048	0·8074	—0·5717	0·5760	0·0099				
									F_{slopes}	0	NS**
								Medium and Hard			
									$F_{elevations}$	2·8506	NS**
Hard	9	0·2546	—0·1365	0·0794	—0·5333	0·0064	0·0008				
									F_{slopes}	2·0000	NS*
								Hard and Soft			
									$F_{elevations}$	345·8750	HS*

NS, Not Significant; *S*, Significant; *HS*, Highly Significant; **d.f.*, F_{slopes} : 1, 16 and $F_{elevations}$: 1, 17; ***d.f.*, F_{slopes} : 1, 66 and $F_{elevations}$: 1, 67

waters for males and between medium and hard waters for females. However, there are differences in the elevations between soft and medium waters in females ($F_{1,67} = 7.411$ $P < 0.05$) and soft and hard waters in both the sexes (males : $F_{1,17} = 48.2$ $P < 0.01$) females : $F_{1,17} = 345.875$ $P < 0.001$) suggesting that the metabolic rate of snails does change in these categories of water.

Discussion

Our observations show that there was no significant difference in the metabolic rate of males in the soft and medium, medium and hard waters and of females in the medium and hard waters. The difference in the metabolic rate of females in the soft and medium waters and of both the sexes in soft and hard waters were significant.

Harrison (1968) studying the effects of calcium carbonate concentration on oxygen consumption of the freshwater snail *Biomphalarea pfeifferi* found higher rates of oxygen uptake in medium waters (calcium mean 12 mg/l and bicarbonate mean 35 mg/l) and attributed this to poor buffering of respired carbon dioxide in softer waters. Dussart and Kay (1980) while investigating the relationship between water chemistry and the rate of respiration in several populations of *Lymnaea peregra*, found higher rates in populations from soft water than from medium or hard waters. They concluded that the higher metabolic rate in soft water might be due to a greater metabolic work for uptake of calcium. Continuity between internal and environmental cation reservoirs has been shown for *Lymnaea stagnalis* (Greenaway 1971b) and *Biomphalaria glabrata* (Thomas & Lough 1974).

Thomas and Lough (1974) suggested that at low environmental concentrations the uptake of ions by active transport was necessary and at high environmental concentrations there would be a net influx into the animal. Dussart and Kay (1980) also suggested that the animals might have to do more metabolic work, at higher or lower environmental concentrations, either to eliminate or absorb calcium. Thus it is possible that the high metabolic rate of *Bellamya bengalensis* in hard water may be due to greater metabolic work to remove excess of calcium entering the body. In soft waters the animals might have to do more metabolic work to take up calcium by active transport only if the environmental calcium levels falls below a certain critical level. Perhaps, above this critical level, the animals may take up ions by passive transport (Prosser 1973) without any increase in metabolic work.

The absence of significant difference ($P > 0.05$) in the metabolic rate of males in soft and medium water, and its presence in the females may be due to differences in the sensitivity of the two sexes to external ion concentrations attributed to varying critical levels. This might be an additional possible explanation of Dussart and Kay's results and suggests more detailed study on the critical levels of these ions which may show both inter- and intra-specific variations.

Acknowledgements

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Correlation between Intermoult Cycle and Ovarian Growth in the Freshwater Prawn, *Macrobrachium kistnensis*

R SAROJINI, R NAGABHUSHANAM and C V R REDDY
Department of Zoology, Marathwada University, Aurangabad 431004

(Received 29 March 1984; after revision 31 June 1984)

A correlation between intermoult cycle and ovarian growth in the prawn, *Macrobrachium kistnensis*, is described, based mainly on statistical studies of the natural population. The ovaries were small, flat, transparent, and whitish in colour at intermoult stage B_1 and slightly greenish at stage B_2. Eggs on the pleopods of ovigerous females were green in colour in stage B_2. The ovaries were light green to deep green in stage C. Fully grown ovaries were found in stage D_0 to D_2 and A, as indicated by the highest gonadal index. Some females in stage A_2 bore spermatophores, indicating that they had mated soon after moult. Egg laying occurred in stage $A_2 - B_1$, and hatchig of larvae between D_0" and D_1". All the intermoult stages were evident in the nonovigerous females. Most of the individuals were in stage C (about 50%); the other stages were shorter in length.

Key Words: **Intermoult cycle**, **Ovarian growth**, *Macrobrachium kistnensis*

Introduction

Reproduction and moulting are two synchronous physiological processes as mating and spawning are preceded by a moult in most of the crustaceans (Patel & Crisp 1961).

The basic schemes of reproduction are met within Crustacea depending upon the possibilities of number of reproductive cycles within one intermoult cycle. While most of the crustaceans have only one reproductive cycle within one intermoult cycle, some others have two or even more such cycles.

Several indirect evidences indicate that the correlation between moulting and reproduction is the same in all the prawn species. Further, there may be one or more reproductive cycles in a single intermoult cycle. For instance penaeid prawns can spawn several times during one complete moult cycle (*Parapenaeopsis stylifera* and *P. hardwickii*—Joshi 1980; *Penaeus indicus*—Emmerson 1980). Since no findings correlating precisely moulting and reproduction in caridean prawns are available, it was thought fit to undertake such a study in the freshwater caridean prawn, *Macrobrachium kistnensis*.

Material and Methods

Specimens of *Macrobrachium kistnensis*

Tiwari 1952, were collected with a hand net from Kham River, about 15 miles south of Aurangabad. Three collections were made at 2 weeks interval (on January 18, 31 and February 11 respectively) to ascertain the inexistance of synchrony of exuviations. Total length of animals was measured from the tip of the rostrum to the end of the telson and adjusted to the nearest millimeter. Fresh weight were measured to the nearest 0.01 g. Intermoult stages were determined as soon as possible after collection (within 24 hr) by microscopical observation of apical setae on the scaphoscerite or on exopodite from the uropod, according to Drach's method (developed by Drach 1944, Drach & Techernigovtezeff 1967, Techernigovtzeff 1965). After careful dissection, the fresh weight of the ovary was obtained and the ovarian index (OI) was calculated. The females that bore no eggs on the pleopods are termed here as "females", including immature ones and the others are termed as the "ovigerous females".

Results

Main Characteristics of the Population

The total length of the females (figures 1 and 2) was between 26.5 and 56mm, and that of ovigerous females between 40 and 61 mm. A few males were also considered for comparison only, and they were 30 to 49 mm long. The largest ovigerous females weighed more than 3 g (maximum 3.51 g for 60 mm). Most of the females were smaller than minimum sized ovigerous females. They were, therefore, in vitellogenesis and might soon have to undergo their pubertal moult.

The morphological aspects of intermoult stage in *M. kistnensis* were similar to those reported in some Palaemonid prawns, and these were not uniformly distributed (figure 3). Ovigerous females were found only between stages B_1 and D_1", while females were present during all the stages. Comparision of (tables 1 and 2) indicates that the distribution of the stages at a 12 days interval was quite similar.

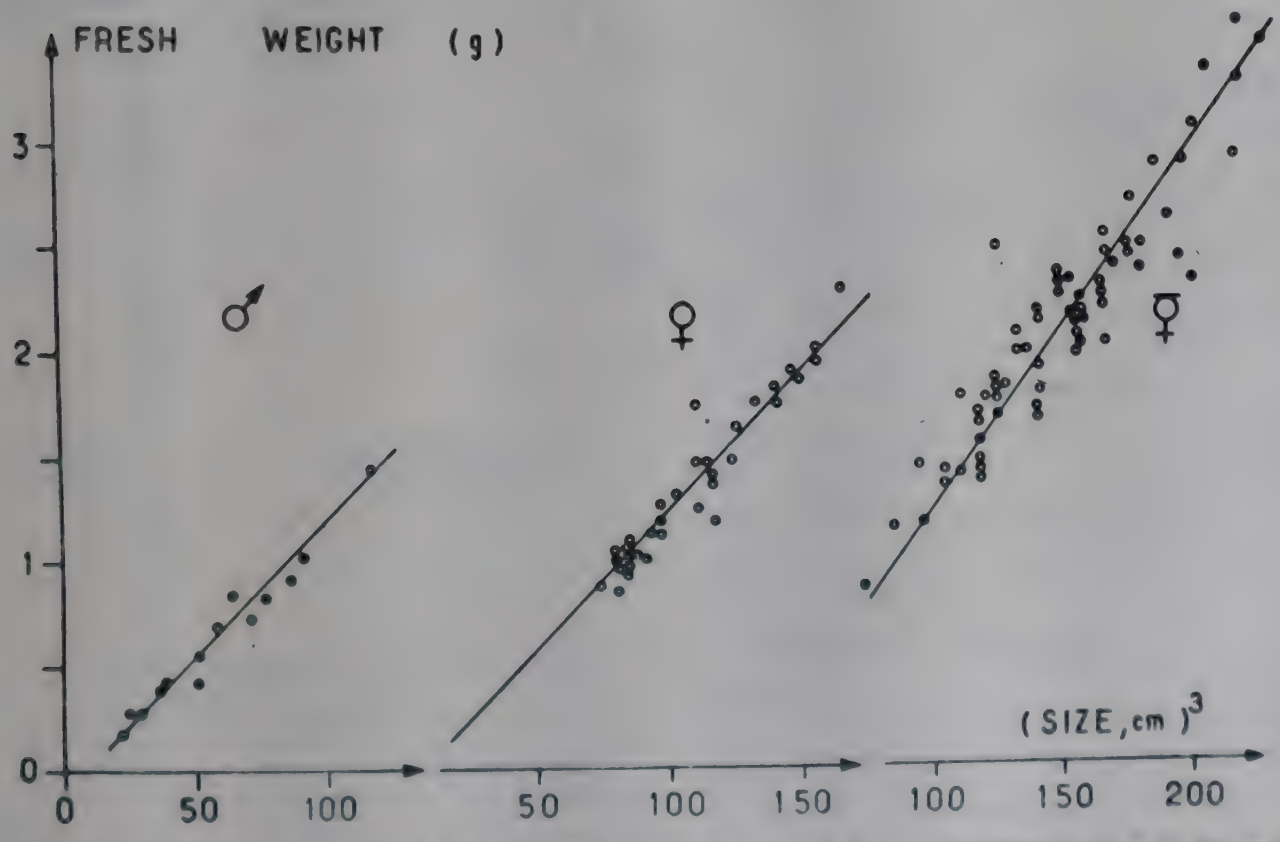

Figure 1 Relationship between size and fresh weight in males (♂, n = 14) female (♀, n = 39), and ovigerous females (♀, n = 65) of prawn, *M. kistnensis*

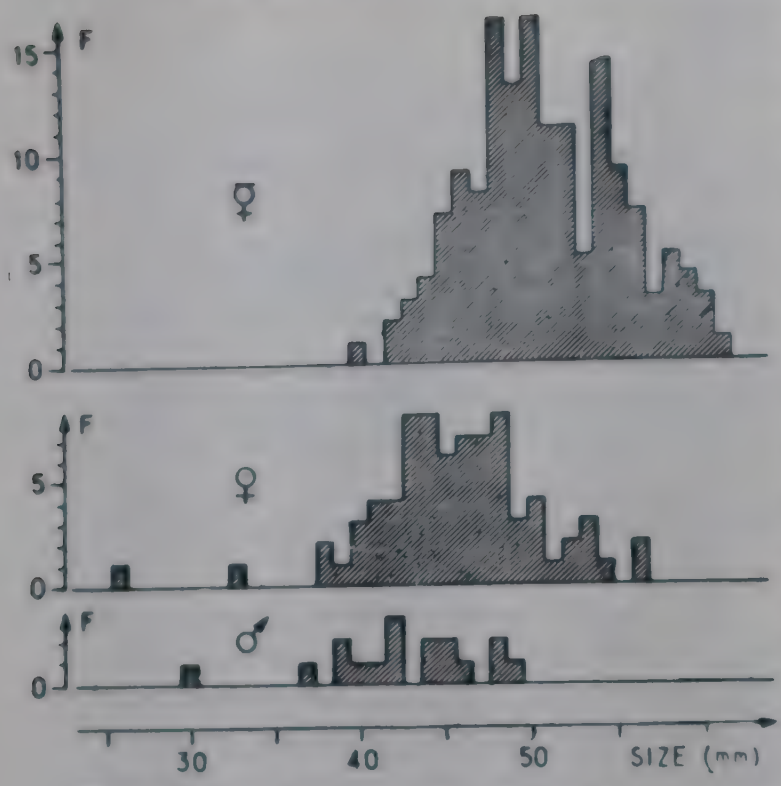

Figure 2 Size distribution in males (♂), females (♀), and ovigerous females (♀) of *M. kistnensis* (classes of 1 mm; F = absolute frequency; 16 males from 30 to 49 mm, 76 females from 26 to 56 mm and 154 ovigerous females from 40 to 61 mm)

Based on the frequency of intermoult stages, the relative length of each stage was worked out, the longest one being the more frequent one.

Correlation between the Intermoult Stages and the Growth of the Ovary

In stage B, the ovary was small, flat, transparent, whitish at the beginning and sometimes slightly greenish later; with very low ovarian index (0.64) (figure 4) and bore eggs on the pleopods. In stage C, the ovary grew gradually and the colour turned from whitish grey to light green. The ovarian index rose progressively up to 1.2. Correlatively, the digestive gland decreased in size, due to mobilization of reserves for ovarian maturation. In stages D_0 to D_1, the ovary continued its growth and the ovarian index reached to 2 in D_0, 5 in D_1 and 10 in $D_2 - D_4$. Ovigerous females were not found after D_1''. In stages between D_3 to D_4 and between A_1 to A_2, the ovary was dark green and often enormous, the ovarian index ranged between 8 to 12. This means that the ovary is more than 10% of the total weight of animal. Spawning occurred at the end of A_2 or at the beginning of B_1. Recently moulted females (in stage A_2) were sometimes found impregnated, bearing spermatophores between coxae of 5th pair of walking legs and 1st pair or pleopods.

Discussion

Interpretation of the Results

Copulation occurred in stage A_1 as indicated by the spermatophore bearing females. The absence of ovigerous females in A, D_2 and D_4 indicated that spawning took place in stage B_1 (or late A) and that larvae hatched between D_0'' and D_2''. Females between D_2 and D_4 stages had egg membranes on pleopods; these post-ovigerous females often had a large ovary, indicating the preparation for a new reproductive cycle during next intermoult.

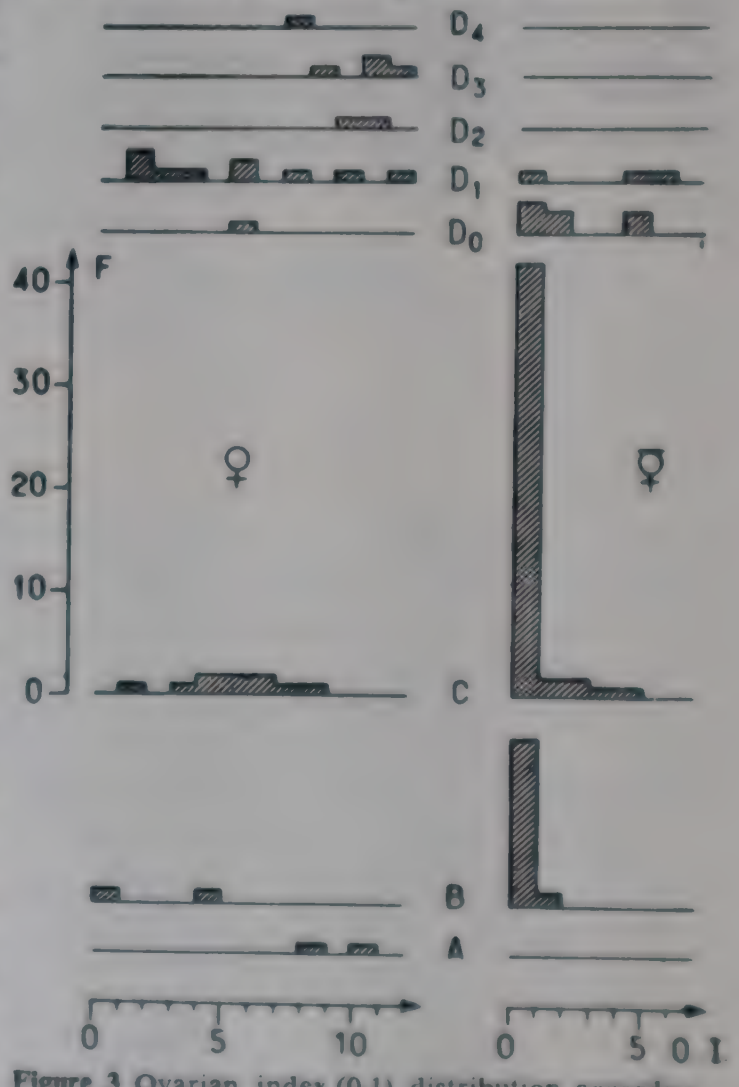

Figure 3 Ovarian index (0.1) distribution according to intermoult stages (A, B, C, D_0, D_1, D_2, D_3, D_4) in January

Table 1 *Frequency of intermoult stages in females and ovigerous females collected on January 31, 1980*

Stage	A	B	C	D_0	D_1	D_2	D_3	D_4	Total
Females	3	7	18	1	10	10	5	1	47
Ov. females	0	11	30	7	3	0	0	0	51
Total	3	18	48	8	13	2	5	1	98
%	3·06	18·37	48·98	8·16	13·27	2·04	5·10	1·02	100

Table 2 *Frequency of intermoult stages in females and ovigerous females collected on February* 11, 1980

State	A	B	C	D_0	D_1	D_2	D_3	D_4	Total
Females	3	6	8	8	6	3	1	0	35
Ov. females	0	11	42	8	4	0	0	0	65
Total	3	17	50	16	10	3	1	0	100
%	3	17	50	16	10	3	1	0	100

January and February appeared to be the reproductive season since most of the mature female ovaries had oocytes in secondary vitellogenesis. The females with a large ovary in stage C were immature (between 45 and 50 mm) with no ovigerous setae on these pleopods; these females had just started their first reproductive period.

Correlation between Moult Cycle and Reproductive Cycle

Female *M. kistnensis* were in ovigerous condition in January and most of them showed intense vitellogenesis and reproductive period extended to at least March. Four types of moult cycles were distinguished in females, in relation to reproduction (Descounturelle 1976), sexual rest (no vitellogenesis or weak one), intense vitellogenesis (preceding the first egg laying), incubation with intense vitellogenesis (preparation of the next spawnig); incubation without vitellogenesis (at the end of reproductive period). Only the second and the third kinds of cycles occurred in January in *M. kistnensis*.

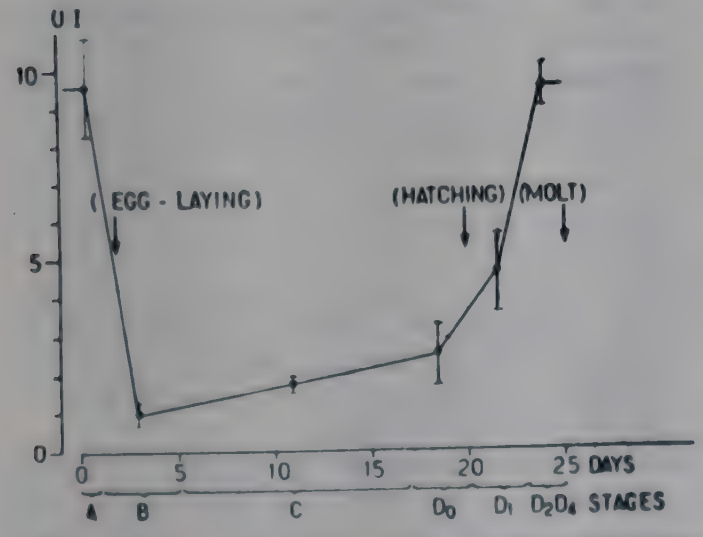

Figure 4 Ovarian development of mature prawns 40 mm or more: 0.1 = ovarian index)

Criteria for Identification of Intermoult Stages and Their Duration

The method of determination of respective length of the different intermoult stages from their frequency was first used by Drach (1944). His results on *Palaemon serratus* are not very different from ours on *M. kistnensis*.

Estimation of the relative duration of the intermoult stages (table 3) compared with those of various Crustacea (Reake 1975) shows that *M. kistnensis* has quite long C and D_0 stages. These values should be confirmed by laboratory reading.

Table 3 *Mean proportion of the different intermoult stages in* M. kistnensis (*animals in period of vitellogenesis*) *corresponding theoretical duration in days based on percentages, for 20 and 25 days*

Stages	A	B	C	D_0	D_1	D_2	D_3	D_4	Total
Females	6	13	26	9	16	5	6	1	82
Ov. females	0	22	72	15	7	0	0	0	116
Total	6	35	98	24	23	5	6	1	198
	3·03	17·68	49·48	12·12	11·62	2·53	3·03	0·51	100
Theoretical	0·8	4·4	12·4	3·0	2·9	0·6	0·8	0·1	25
Duration	0·6	3·5	9·9	2·4	2·3	0·5	0·6	0·1	20

Intermoult Stages and Ovarian Development

Correlation between intermoult stages and stages of ovarian maturation in decapod crustaceans have rarely been reported. Copulation in *Leander serratus* takes place before egg deposition and after parturialmoult (Yoshida 1952). Sand shrimp, *Crangon allmani* moults prior to the extrusion of eggs (Allen 1960). Freshly berried *Caridina weberi* were also found to have recently moulted (Chinnayya 1968). Thus, in these carideans, egg laying might occur in stage A. Ovarian growth starts again immediately after egg laying. While in most shrimp, copulation occurs just after moulting, this is not the general rule for other Crustacea. It may take place in moulted females and in berried females (Patel & Crisp 1961, Hazlett 1975). For the reproductive period, Drach (1965) noted in *Palaemon serratus* that the intermoult cycle begins at egg laying and ends shortly after hatching. In *M. kistnensis*, the phenomenon may be exactly the same as in *P. serratus*, except that hatching occurs much earlier.

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Aminotransferases and Alkaline Phosphatase Activities of Serum, Liver and Skeletal Muscle in Albino Rats Infected with *Trichinella spiralis*

N HAZRA*, G MAJUMDAR*, C R MAITY† and B MANDAL‡

**Parasitology Laboratory, Zoology Department, Burdwan University, Burdwan 713 104*

(Received 25 September 1984; after revision 14 February 1985)

Aminotransferases and alkaline phosphatase activities of serum, liver and skeletal muscle, studied in *Trichinella*-infected albino rats on 2, 5, 13, 21, 28, 35, 50 and 60 days of postinfection, showed that though the activities of serum and liver increased significantly until 13 dyas of postinfection these remained unchanged afterwards as compared to those of control. On the contrary, the enzymatic activities of skeletal muscle of the infected hosts did not undergo any appreciable change during early days of postinfection but increased gradually afterwards.

Key Words: *Trichinella spiralis*, **Aminotransferases, Alkaline phosphatase**

Introduction

The incidence of trichinosis, a parasitic infection caused by *Trichinella spiralis* has recently increased due to increased consumption of pork and pork products (Damodar & Narayana Rao 1982). In this parasitic infection, growing larvae actively exchange several metabolities with the host and as such some disturbances in metabolic processes as well as in the enzymatic activities do take place. *T. spiralis* has been reported to interfere with the phenomenon of carbohydrate metabolism in host (Brand 1966, Saz 1969). In a recent study (Hazra et al. 1984a), we have shown that lipid metabolism of host is influenced during late stages of *Trichinella* infection. Certain changes in protein metabolism is also noted in *Trichinella* infection (Dawes 1963). Read (1970) reported that some cellular or subcellular damages or derangements occurred during the penetration of *Trichinella* larvae into different tissues. Thus, such derangements may be responsible for the changes in the carbohydrate, protein or lipid metabolism and in the activities of some enzymes. Our studies (Hazra et al. 1984b,c) have also shown that phosphohexose isomerase and aldolase activities increase during *Trichinella* infection and the estimation of these enzymes may be

†Biochemistry Department, Burdwan Medical College, Burdwan 713104
‡Chemistry Department, B N Mahavidyalaya, Itachuna P.O., Hooghly, West Bengal

helpful for the diagnosis of severity in trichinosis. The present investigation was extended to study the alteration of aminotransferases and alkaline phosphatase activities of serum, liver and skeletal muscle in *Trichinella*-infected albino rats to ascertain whether these enzymes could be used as index of severity in trichinosis.

Materials and Methods

Fourty-eight male albino rats (inbred in our laboratory) weighing about 50–60 g were divided into two groups, control or non-infected (C) and infected (T), of 24 animals each. Animals of T group were infected with *Trichinella spiralis* larvae (1000–1200 larvae per animal) following the procedure described earlier (Hazra et al. 1984d). The strains of *Trichinella spiralis* obtained from the School of Tropical Medicine, Calcutta, were maintained in laboratory-bred albino rats. Autopsied rats, less than 7 months postinfection, were subjected to pepsin digestion at 37°C and the larvae were collected (Hazra et al. 1984d). The animals were individually caged and maintained on standard laboratory diet (Mandal et al. 1982) and water *ad libitum* until experimentation. Three infected animals were sacrificed at a time and on 2, 5, 13, 21, 28, 35, 50 and 60 days of postinfection along with the same number of controls side by side. Blood, liver and skeletal muscles were collected and immediately placed in chilled container for experimentation. Alanine aminotransferase and aspartate aminotransferase activities were measured by the method of Reitman and Frankel (1957), alkaline phosphatase by Bessey et al. (1946) and protein by Lowry et al. (1951). Statistical analysis was done by Students's t-test.

Results

Alanine aminotransferase activity of serum and liver increased significantly upto 13 days of postinfection but afterwards it remained unchanged in the infected hosts (table 1). In contrast, the enzymatic activity of skeletal muscle did not undergo any noticeable alteration until 5 days of postinfection, afterwhile it raised significantly with the days of postinfection (table 1). Aspartate aminotransferase activity of serum and liver of the infected hosts also showed a significant rise until 13 days of postinfection and thereafter the activity remained unchanged as compared with the controls (table 2). Aspartate aminotransferase activity of skeletal muscle of the infected hosts did not show any appreciable alteration until 13 days of postinfection. The activity then increased gradually up to 60 days of postinfection (table 2). Table 3 shows the alkaline phosphatase activity of serum, liver and skeletal muscle of the infected hosts and those of controls. Alkaline phosphatase activity of serum and liver rose significantly until 13 days after infection and thereafter the activity remained unchanged. On the contrary, the enzymatic activity of skeletal muscle of the infected hosts remained unaltered until 13 days of postinfection but afterwhile it increased gradually.

Discussion

The results of the present study show that the aminotransferases and alkaline phosphatase activities of serum and liver of the *Trichinella* infected hosts rose significantly at early days of infection but at late days the activities remained unchanged. It is reported (Brand 1966) that at early days of infection almost all the *T. spiralis* larvae are found in the mucosa of the small intestine. Therefore, during the intestinal phase of infection, striking signs and symptoms of this pathological process may appear. Thereafter, it is not unusual for the migrating larvae to invade the liver tissue early in the

Table 1 *Alanine aminotransferase activity of serum, liver and skeletal muscle of* Trichinella *infected albino rats at different periods of postinfection* ($n=3$, ± *Standard error*)

Days of post-infection	Serum (i.u./litre/min)		Liver (μ mole pyruvate/mg protein/hr)		Muscle (μ mole pyruvate/mg protein/hr)	
	A	B	A	B	A	B
2	6·1±0·42	8·8±0·51[a]	9·4±0·56	12·1±0·82[a]	4·3±0·11	4·8±0·42
5	6·5±0·36	9·6±0·48[b]	9·2±0·45	13·4±0·91[b]	4·5±0·20	4·6±0·31
13	6·4±0·42	9·8±0·54[b]	9·6±0·61	15·6±0·88[c]	4·6±0·25	5·8±0·30[a]
21	6·8±0·48	7·1±0·62	9·5±0·74	10·1±0·58	4·8±0·12	6·7±0·31[b]
28	6·6±0·51	6·9±0·54	9·2±0·81	9·8±0·64	4·4±0·34	7·8±0·45[c]
35	7·1±0·36	6·9±0·48	10·1±0·71	10·2±0·84	4·6±0·25	9·2±0·42[d]
50	7·0±0·58	6·8±0·53	9·9±0·48	9·8±0·62	4·3±0·22	9·8±0·36[d]
60	6·6±0·36	6·7±0·48	9·5±0·85	10·1±0·81	4·8±0·32	10·6±0·42[d]

Values for the infected animals (B) were significantly different from those for the corresponding controls (A) : a_p <0·05; b_p <0·01; c_p <0·002; d_p <0·001

Table 2 *Aspartate aminotransferase activity of serum, liver and skeletal muscle of* Trichinella *infected albino (rats at different periods of postinfection* ($n=3$, ± *Standard error*)

Days of post-infection	Serum (i.u./litre/min)		Liver (μ mole pyruvate/mg protein/hr)		Muscle (μ mole pyruvate/mg protein/hr)	
	A	B	A	B	A	B
2	10·4±0·62	12·6±0·54[a]	5·4±0·36	7·4±0·62[a]	7·1±0·35	7·4±0·44
5	10·8±0·56	14·9±0·68[b]	5·6±0·41	8·4±0·58[b]	7·6±0·46	7·5±0·38
13	9·6±0·66	15·2±0·54[c]	5·8±0·48	9·6±0·34[c]	8·0±0·54	7·6±0·45
21	9·8±0·54	9·6±0·42	6·1±0·46	5·8±0·51	7·5±0·26	8·8±0·30[a]
28	9·8±0·48	9·8±0·45	5·4±0·48	5·6±0·35	7·3±0·31	9·4±0·42[b]
35	9·9±0·58	9·6±0·43	6·2±0·61	6·0±0·34	7·6±0·41	10·9±0·38[c]
50	10·3±0·68	9·8±0·64	6·1±0·52	5·9±0·60	7·3±0·35	11·8±0·50[d]
60	10·1±0·66	10·1±0·59	5·5±0·44	5·8±0·46	8·1±0·48	13·5±0·46[d]

Values for the infected animals (B) were significantly different from those for the corresponding controls A) : a_p <0·05; b_p <0·01; c_p <0·002; d_p <0·001

Table 3 *Alkaline phosphatase activity of serum, liver and skeletal muscle of* Trichinella *infected albino rats at different periods of postinfection* ($n=3$, ± *Standard error*)

Days of post-infection	Serum (King Angstorn Unit/100 ml)		Liver (μg p-nitrophenol/ mg protein/hr)		Muscle (μg p-nitrophenol/ mg protein/hr)	
	A	B	A	B	A	B
2	8·1±0·54	9·2±0·62	10·4±1·11	14·3±0·91[a]	5·2±0·36	5·5±0·24
5	7·6±0·45	12·5±0·81[b]	10·2±0·92	16·6±0·82[b]	5·8±0·45	6·2±0·38
13	7·8±0·65	12·6±0·64[b]	9·8±0·64	15·9±0·85[b]	5·4±0·34	6·0±0·42
21	7·5±0·44	8·1±0·35	9·6±0·88	10·8±0·92	5·6±0·41	7·4±0·36[a]
28	8·2±0·36	8·2±0·62	10·2±0·69	10·6±0·94	6·1±0·38	9·6±0·42[b]
35	7·8±0·54	8·3±0·49	9·8±0·92	10·5±0·64	5·6±0·41	9·4±0·34[b]
50	8·2±0·45	8·5±0·68	10·1±0·61	9·8±0·83	6·2±0·35	11·3±0·54[c]
60	7·6±0·36	7·9±0·45	9·5±0·58	9·6±0·75	6·0±0·41	12·6±0·58[c]

Values for the infected animals (B) were significantly different from those for the corresponding controls (A) : a_p <0·05; b_p <0·01; c_p <0·001

infection, but few have been found there (Sinha 1977, Hazra 1982). Probably the invasion of the parasitic larvae into the liver results some hepatocellular damage or derangement. It seems probable that this hepatocellular damage or derangement is responsible for the increase in the activities of aminotransferases and alkaline phosphatase in serum and liver. Though it is reported that after 2–5 days of infection almost all the larvae are found to migrate into the muscle leaving all other organs (Brand 1966) but our study has shown that the aminotransferases and alkaline phosphatase activities of serum and liver fail to reach normal until atleast 13 days of postinfection. This residual effect is probably due to toxic products excreted by the parasites.

Probably at early stage of infection, invasion of parasitic larvae into the muscle did not occur which explain for the unaltered enzymatic activities of skeletal muscle in the infected hosts. But at the late stages, almost all the growing parasites penetrate the skeletal muscle and subsequently muscle cells are damaged (Read 1970). Damaged muscle cells release aminotransferases and alkaline phosphatase enzymes, so their concentrations are increased in the skeletal muscle tissue.

Thus, the present study revealed that the measurement of aminotransferases and alkaline phosphatase activities at different periods of *Trichinella* infection are of considerable significance for the diagnosis of severity in trichinosis, which is yet to be studied.

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Sciatectomy and Induced Exercise on the Hepatic Nitrogen Metabolism of Dog, *Canis domesticus*

Y DHANANJAYA REDDY, K VENKATARAMI REDDY, O RAMAKRISHNA*, S GOVINDAPPA and P REDDANNA

Department of Zoology, Sri Venkateswara University, Tirupati 517 502

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Hepatic nitrogen metabolism was analysed during sciatectomy and induced exercise to sciatectomized dogs, *Canis domesticus*. The muscular atrophy induced through sciatectomy resulted in hepatic protein degradations and accumulation of ammonia which was averted by induced exercise.

Key Words: **Liver, Sciatectomy, *Canis domesticus*, Hepatic metabolism, Muscular atrophy, Muscular work overload**

Introduction

Among all tissue metabolic loads exercise or work performance is considered to be the most important one (Herbinson et al. 1973, Pette et al. 1973, Angeli et al. 1979). Prolonged and exhaustive whole body exercise lead to trained conditions of the skeletal muscle (Penman 1960, Holloszy et al. 1975) Electrical stimulation of muscle and exercise alter the metabolism of various tissues in the carbohydrate metabolism in the body (Gutmann 1962, Hickson et al. 1977). The *in vivo* muscular electrical stimulations increased the activity levels of oxidative enzymes of most of the tissues in the body (Reddanna & Govindappa 1978). Thus investigations on the metabolism of other tissues during induced muscular overwork revealed the metabolic inter-relationship between muscle and other tissues in the body (Venkatarami Reddy et al. 1984a,b,c). However, there has been meagre information on the hepatic metabolism during muscular atrophies and on application of electrical treatment to the atrophying muscles. Hence the present study was undertaken.

Material and Methods

Material

Twelve healthy male mongreal dogs, *Canis domesticus*, age 3–4 years and weighed 11–16 kg were randomly divided into three groups as follows:

Group I : Normal sham operated dogs designated as "Controls" (C)

Group II : Experimental animals with unilateral sciatic denervation for a period of 15 days and termed as "denervated" (D)

*Professor of Surgery and Radiology, College of Veterinary Sciences, Tirupati 517 502

Group III : Experimental animals with gastrocnemius muscle of denervated leg electrically stimulated for 15 days, called as "denervated stimulated" (S)

The muscular sciatectomy and electrical stimulations were conducted as described earlier (Venkatarami Reddy et al. 1983, 1984a,b). On the day sixteenth after stimulation, the dogs were sacrificed and liver dissected out in cold condition (at 4°C) and homogenates (5% W/v) in required media were prepared. The homogenates were centrifuged at 4000×g and the cell free extracts were used for the enzyme assays and estimation of metabolites.

Methods

Protein content was estimated by the method of Lowry et al. (1951). Glutamine (Colowick & Kaplan 1957), ammonia (Bergmeyer 1965), urea (Natelson 1971), free amino acid and the levels of protease activity (Moore & Stein 1954), alanine and aspartate amino transferases (Reitman & Fraenkel 1957) and the activity level of glutamate dehydrogenase (Lee & Lardy 1965) were determined by the methods given in the parentheses.

Results and Discussions

The data presented in table 1 reveals the pattern of hepatic tissue protein metabolism associated with muscular atrophy as well as during the electrotherapy of the atrophying muscles.

When the muscle was subjected to denervation atrophy through sciatectomy, hepatic tissue showed considerable changes in the protein metabolism, which can be attributed to the relationship between hepatic tissue and atrophying muscle. The protease activity of the hepatic tissue was significantly increased over the control under denervation atrophy of the muscle. The atrophying muscle releases the calcium into the plasma (Yakovlev 1956) and the calcium being an activator of proteases, the hepatic proteases seem to have been activated through the mediation of calcium. Consequently, there was depletion in soluble and structural protein fractions of the hepatic tissue. However, the amino acid content was significantly decreased, which indicates their mobilization into the metabolic activities. The activity levels of GDH and AAT which represent the oxidative deamination of amino acids were elevated over the control levels indicating the activation of amino acid oxidations in the tissues and the same can be substantiated by the accumulation of the free ammonia in the tissue. The levels of both urea and glutamine were markedly depleted over the control levels which indicates the derangement in the mobilization of free ammonia into these components, which might mark the decreased efficiency of ammonia detoxificatory mechanisms of the hepatic tissue of the animals with atrophying muscles.

However, the situation of hepatic tissue mechanism was different when the atrophying muscles were subjected to electrical stimulation. Under the conditions of electrotherapy, the atrophying muscle will be subjected to activity, which promotes release of calcium into the plasma (Yakovlev 1956). Similarly, branched chain amino acids were released from the working muscle (Usami 1957, Rozogkin 1959). These factors activate the proteolysis (Grove et al. 1969) and hence the hepatic tissue protease activity was significantly increased. However, the soluble and structural protein fractions had no change from the control levels, indicating the possibility of activation of synthetic phase of protein metabolism. The tissue RNA content was elevated under the electrical stimulation of the muscle (Grove et al. 1969). Hence when the deneravation muscle was subjected to electrical stimulation the hepatic tissue protein synthesis might have been

Table 1 *Levels of soluble proteins, structural proteins, protease activity, free amino acids, alanine aminotransferase (AlAT) aspartate amino transferase (AAT), glutamate dehydrogenase (GDH), glutamine, ammonia and urea in hepatic tissue of control (C) and experimental (D, S) animals*

Parameter	Control (C)	Experimental	
		Denervated (D)	Stimulated (S)
Soluble proteins (mg/g wet wt)	107·10±10·00	81·09± 2·23 —24·29 $P < 0·001$	115·46± 9·25 + 7·81 NS
Structural proteins (mg/g wet wt)	72·65±5·10	60·19± 2·35 —17·15 $P < 0·001$	74·21± 5·12 + 2·15 NS
Protease activity (μ moles/mg protein/h)	0·115±0·004	0·216± 0·001 +87·83 $P < 0·001$	0·231 ± 0·006 + 100·87 $P < 0·001$
Free amino acids (μ moles/g wet wt)	9·22±0·71	4·99± 0·99 —45·89 $P < 0·001$	12·19± 1·09 +32·21 $P < 0·001$
AlAT (μ moles of pyruvate/mg protein/h)	0·911±0·09	0·982± 0·09 + 7·79 NS	0·962± 0·07 + 5·59 NS
AAT (μ moles of pyruvate/mg protein/h)	0·68±0·02	0·85± 0·07 +25·00 $P < 0·001$	0·71± 0·07 + 4·42 NS
GDH (μ moles of formazan formed/mg protein/h)	0·171±0·01	0·234± 0·03 +36·84 $P < 0·001$	0·088± 0·002 —48·54 $P < 0·001$
Glutamine (μg moles of ammonia/g wet wt)	16·51±1·20	8·13± 0·08 —50·76 $P < 0·001$	13·86± 0·97 —16·05 $P < 0·001$
Ammonia (μ moles/g wet wt)	1·57±0·05	2·69± 0·02 +71·34 $P < 0·001$	0·95± 0·09 —39·49 $P < 0·001$
Urea (mg/g wet wt)	0·91±0·08	0·67± 0·05 +26·37 $P < 0·001$	1·32± 0·18 +45·05 $P < 0·001$

Each value is mean of six observations (Mean±S D);
+ and — indicate per cent increase and decrease over the control;
P denotes level of significance, NS=non significance

activated. Thus in this animal the hepatic tissue protein fractions seem to be in dynamic turnover with activated proteolysis on one side and synthesis on the other, leading to non significant change in the levels of soluble and structural proteins. GDH activity was inhibited markedly leading to decreased free ammonia level. Urea content was elevated indicating the possible activation of urea synthesis and the consequent mobilization of free ammonia into the urea formation. Thus the electrotherapy to denervated muscle was leading to relieve the ammonia toxicity of hepatic tissue through the activation of detoxificatory mechanisms.

Hence, it can be concluded that the hepatic tissue was exposed to ammonia toxicity in the presence of atrophying muscles which was relieved by the application of electrical stimulations to the atrophying muscles.

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Limnological Studies of Thermal Springs of Bihar, India

K S BILGRAMI, J S DATTA MUNSHI, R N YADAVA and B N BHOWMICK
Post-Graduate Departments of Botany/ Zoology, Bhagalpur University, Bhagalpur 812007

(Received 27 July 1984; after revision 19 January 1985)

In the two thermal springs Bhimbandh (63–39°C) and Rishikund (46–31°C) located in Munger district, Bihar the temperature at the source of emergence as well as at other points was constant throughout the year. With the fall of temperature, the *p*H, dissolved O_2 and alkalinity increased, whereas the silicate content declined. Some rare gases like helium and argon were also discharged alongwith hot water at the source. At temperature above 60°C only blue-green algae and bacteria were recorded. The species diversity was very limited in this range. Green algae and diatoms were recorded only at lower temperatures. Below 46°C the frequency of blue-greens declined and these totally disappeared below 41°C. Chlorophyceae and Bacillariophyceae appeared below this temperature. Protozoans, nematodes rotifers, and insects species were recorded between 38 and 45°C while fishes were found below 45°C.

Key Words: **Limnological survey, Rare gases, Thermal springs**

Introduction

In recent years considerable attention has been paid to the ecological aspects of thermal springs notably by Gonzalves 1947, Thomas et al. 1965, Prasad & Srivastava 1965, Vashist 1967, Patel 1974 who have studied the taxonomy of thermophilic algae. Jana (1977, 1978), Jana and Sarkar (1982), Saha et al. (1978) as well as Saha and Datta Munshi (1979) have studied some limnological aspects of thermal springs. However, very little work is known in the thermal springs of Bihar which lie mainly in the Southern belt of the state. It is hoped that the present limnological account of two hot springs Bhimbandh and Rishikund located in Munger district of Bihar would fill this gap. The study was conducted from October, 1981 to September, 1983.

Materials and Methods

The algal samples were collected at monthly intervals and preserved in 4% formalin. Their frequency was measured by species count method using haemocytometer. Physico-chemical analysis of water was carried out by the methods recommended

by APHA (1975). Total bacterial density was measured by plate count method using nutrient agar and total coliform bacteria were determined by multiple tube dilution technique using MacConkey's broth of single and double strength (APHA 1975). Further, IMViC and other biochemical tests were done according to Sirockin and Cullimore (1969) and Starr et al. (1981). Free carbon-dioxide, dissolved O_2, bicarbonate alkalinity were measured at the locations while Na, K, Ca, Mg, SiO_2 and phosphate phosphorus were determined in the laboratory. Samples of rare gases were sent to Bhabha Atomic Research Centre, Variable Energy Cyclotron Centre, Calcutta for analysis. The objective of estimating the gases was to ascertain as to how the water reaches such a high temperature, below the earth crust. Their presence shows that heat is generated due to radioactive substance like K_{40}.

Bhimbandh and Rishikund thermal springs are situated in the reserve forest area of Kharagpur hill range of Munger district (lat. 25°.3′N, long. 86°25′E). At Bhimbandh hot water comes out from numerous mountain fissures and it forms a stream which subsequently joins a cold stream (figure 1). The water of the stream cools down slowly and develops a gradient from 63 to 39°C along a stretch of about 1.5 Km. The bed of the stream is covered with pebbles having profuse algal deposition over it. The temperature at Rishikund ranges from 46 to 31°C within a distance of about 100 meter (figure 2). The base is mostly stony except in *Kund* (main source) which is muddy at the bottom. Algal growth is quite luxuriant. Six spots at intervals of temperature variation of 5°C were selected at Bhimbandh which had a temperature gradient from 63 to 39°C. Similarly four spots were selected at Rishikund which had a temperature gradient from 46–31°C.

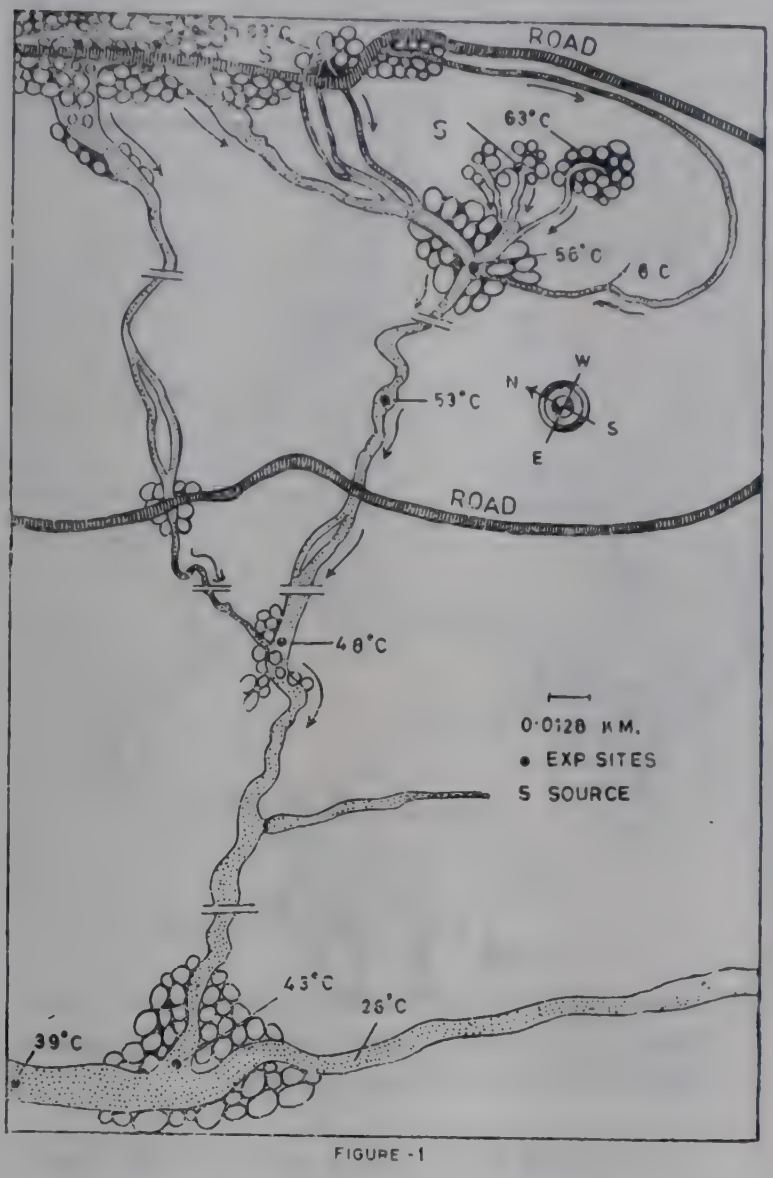

Figure 1 Map showing different temperature gradients of Bhimbandh Thermal Spring.

Results and Discussion

A consistency in temperature at the source was observed (tables 1 & 2). Seasonal variations did not have any effect on water temperature at the source. At other points it was very negligible ($\pm$1°C) as also reported for several other thermal springs too. However, in few thermal springs like Sitakund (India), Geyser (Iceland) and Tsukiji Shokan (Japan) the temperature is known to vary on some occasions even during different hours of the day (Barth 1950, Uzamasa 1965). Both the thermal springs were acidic with low *p*H ranges i.e., 3.9–6.8 and 5.0–6.7 respectively (figures 3 & 4). The rise in *p*H along the thermal gradient was

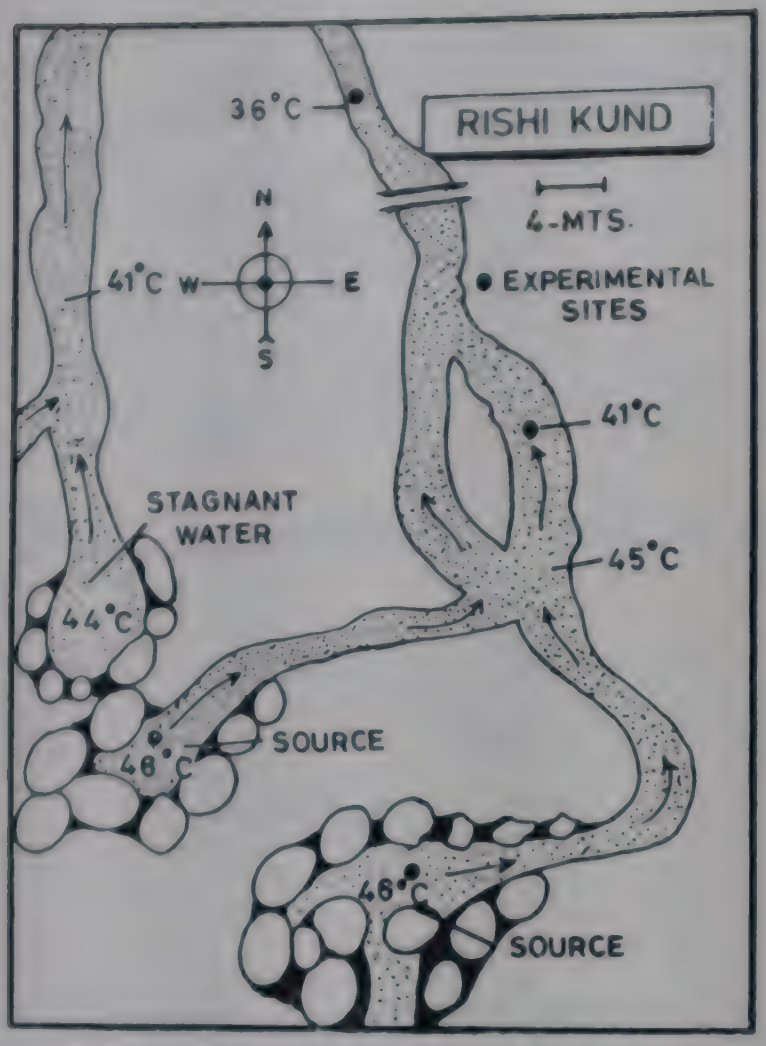

Figure 2 Map showing experimental sites of Rishikund thermal spring with thermal gradient

accompanied by a fall in the level of CO_2. At the source free CO_2 was maximum in both the streams. Rapid fall in free CO_2 down the stream may be attributed to increased periphytonic algae and their photosynthetic activity. Dissolved O_2 at the source was very low both at Bhimbandh and Rishikund. Its concentration increased with the fall of temperature along the stream. This is because of greater diffusion and solubility of atmospheric O_2 at lower temperatures and also due to high photosynthetic activity. This observation supports the findings of Hubbs (1967). Bicarbonate alkalinity was lower near the source at both the places and gradually increased along with the rise of *p*H. Jana and Sarkar (1982) found that the value of silicate remained unaltered at Tantloi and Bakreswar. Silicate concentration however, declined with the fall of temperature both at Bhimbandh and Rishikund. A steep rise in the population of diatoms and Rhizopods at 41°C was the main reason for the fall in silica concentration at lower temperature which had an inverse relationship with the population of diatoms. The phosphate level also declined down the stream both at Bhimbandh and Rishikund suggesting its utilization in the formation of dense algal mats at lower temperatures. Castenholtz (1969) also recorded a fall in phosphate level down the stream at Hunter's hot spring (USA). Brock et al. (1969),

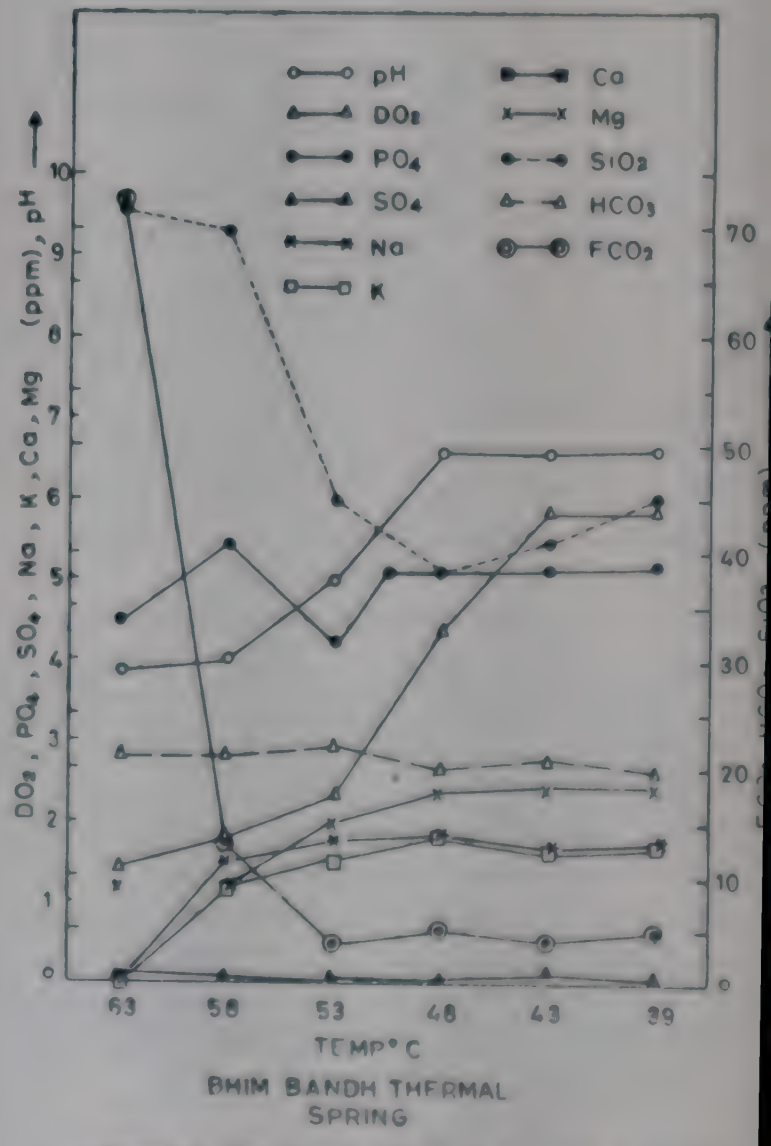

Figure 3 Physico-chemical characters of Bhimbandh thermal spring

Table 1 *Gas analysis at thermal springs of Bhimbandh and Rishikund*

Sources	Temperature at source	Atmospheric temp.	Helium	Argon	Nitrogen %	Oxygen %	Carbon dioxide %
Bhimbandh	63°C	23·5°C	55×10^{-4}	1·2	65·2	6·0	27·6
Rishikund	46°C	24°C	31×10^{-4}	1·3	73·1	5·2	20·4

Table 2 *Distribution of biota in different portions of the effluent channel of Bhimbandh*

Organisms	Temperature (°C)					
	63	58	53	48	43	39
Cyanophyceae						
Synechococcus lividus	+	+	+	−	−	−
S. elongatus	−	+	+	+	+	−
Phormidium africanum	−	+	+	+	+	−
Oscillatoria amphibia	−	+	+	+	−	−
O. germinata	−	+	+	+	−	−
Lyngbya spirulinoides	−	−	+	+	+	−
Scytonema sp.	−	−	+	+	+	−
Nostoc commune	−	−	−	+	+	+
Anabaena sp.	−	−	−	+	+	+
Mastigocladus laminosus	−	−	+	+	−	−
Chlorophyceae						
Oedogonium sp.	−	−	−	+	+	+
Spirogyra sp.	−	−	−	+	+	+
Mougeotia sp.	−	−	−	−	+	+
Ulothrix moniliformis	−	−	−	+	+	+
Closterium sp.	−	−	−	+	+	+
Cosmarium trichyderum	−	−	−	+	+	+
Bacillariophyceae						
Pinnularia sp.	−	−	−	−	+	+
Fragilaria sp.	−	−	−	+	−	−
Gomphonema sp.	−	−	−	−	+	+
Cymbella sp.	−	−	−	−	+	+
Melosira sp.	−	−	−	−	+	+
Fungi						
Chaetomium sp.	−	−	−	+	+	+
Aspergillus flavus	−	−	−	+	+	+
A. fumigatus	−	−	−	+	+	+
Curvularia lunata	−	−	−	+	+	+
Trichoderma sp.	−	−	−	+	+	+
Rhizopus sp.	−	−	−	+	+	+
Protozoa						
Arcella discoides	−	−	−	+	+	+
Euglypha sp.	−	−	−	+	+	+
Vorticella sp.	−	−	−	−	+	+

Contd.

Table 2 *contd.*

Rotifera						
Monostyla sp.	—	—	—		+	+
Brachionus sp.	—	—	—		+	+
Nematoda						
Monunchus macrostoma	—	—	—	+	+	+
Trilobus gracilis	—	—	—	—	+	+
Insecta						
Chironomous sp.	—	—	—	+	+	+
Guignotus pradhani	—	—	—	—	+	+
Pisces						
Channa gachua	—	—	—		+	+
Danio dangila	—	—	—		+	+
Amphibia						
Rana cyanophyctis	—	—	—		+	+

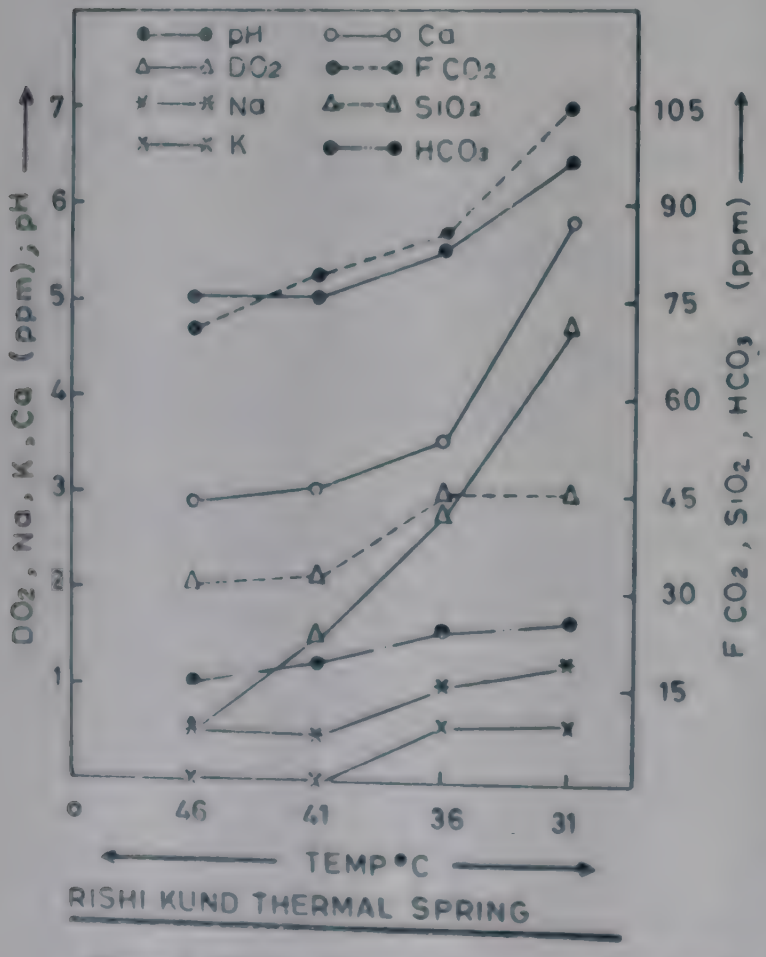

Figure 4 Physico-chemical character of Rishikund thermal spring

however, did not observe any such decrease. Sodium and potassium values also declined with the temperature gradient in both the thermal springs. Increased calcium concentration can be attributed to allochthonous sources mainly due to decomposition of leaf litter which furnishes nutrients like, Na, K, Ca, and Mg. Two rare gases, helium and argon, were found in both the thermal springs (table 1). The amount of CO_2 was fairly high (about 20%). Liberation of helium and argon at the source reflects radioactivity deep in the earth crust. These gases, which emanate along with hot water through narrow fissures, do not seem to have any effect on the organisms, firstly because they are inert and secondly because they escape to atmosphere immediately on coming to the surface.

Myxophyceae constituted 100% population at higher temperatures (51–63°C) at Bhimbandh. At 63°C, *Synechococcus lividus* was the only alga to be encountered but at a slightly lower temperature, say 59°C, few more algae like *S. elongatus*, *Phormidium africanum*, *Oscillatoria amphibia* were recorded. The growth of these algae was most luxuriant between 55 and 50°C. Rich accumulation of algal mat at this temperature was due to total absence of herbivores. Chlorophyceae and Bacillariophyceae were also not recorded at this high temperature. At higher temperatures silicate concentration was more. Similar observations were made

by Round (1970). Peterson (1920) however, recorded diatoms even at 70°C in some thermal springs of Siberia. At 46°C the frequency of blue green algae declined and members of Chlorophyceae and Bacillariophyceae made their appearance. At 41°C the blue-greens were further depleted and the green ones increased substantially. Below 41°C the growth of blue-green algae disappeared and total algal community was occupied by Chlorophyceae and Bacillariophyceae. In Rishikund no species of *Synechococcus* was recorded at 46°C which is the extreme temperature limit of this spring. Chlorophyceae and Bacillariophyceae were dominant at lower temperatures.

Total bacterial density at Bhimbandh near the source was 0.6×10^6/liter at 63°C, and MPN for coliform was 43/100 ml. Both these, however, increased with the fall of temperature (3.1×10^6/liter, MPN 223/100 ml at 51°C) and at 41°C (5.2×10^6/liter, MPN 663/100ml). *Aerobacter aerogenes* type I and II and *E. coli* type I and II were the important coliform bacteria to be encountered at lower temperatures. At higher temperatures some thermophilic bacteria like *Bacillus pasteurii*, *B. anthracis*, *Lactobacillus*, *acidiphilus* were recorded. In Rishikund thermal spring total bacterial density ranged from 2.82×10^6/liter to 4.01×10^6/litre and MPN 174/100 to 218/100 ml at 46 to 41°C. Both these thermal springs are situated in

Table 3 *Distribution of biota in different portions of the effluent channel at Rishikund*

Organisms	Temperature (°C)			
	46	41	36	31
Cyanophyceae				
Lyngbya bipunctata	+	+	+	—
Lyngbya lagerheimii	+	+	—	—
Phormidium tenue	+	+	—	—
Oscillatoria jasorvensis	+	+	—	—
Anabaena circularis	—	+	+	+
Chlorophyceae				
Oedogonium sp.	—	+	+	+
Spirogyra sp.	—	+	+	+
Cosmarium pseudoconnatum	—	+	+	+
C. westii	—	+	+	+
Fungi				
Aspergillus flavus	—	+	+	+
A. niger	—	+	+	+
Curvularia lunata	—	+	+	+
Zooplankton				
Ostracoda				
Stenocypris malcomsoni Insecta	—	+	+	+
Laccophilus anticatus	—	+	+	+
Enochrus sp.	—	+	+	+
Anisops sardia	—	+	+	—
Pisces				
Danio dangila	—	+	+	—

forests where there are large number of herbivorous animals and bacteria (specially the coliforms) appear to come in the system through faecal matter. Few members of Ascomycetes also occur in the springs. The down stream of the channel was moderately populated with protozoa, Rotifera, Nematoda, Insecta, fishes and amphibia at Bhimbandh (table 2). Altogether nine species of invertebrates and three species of vertebrates were collected during the study between 38 to 45°C. In Rishikund zooplankton were not recorded while insects were represented by *Huignotus pradhani* and *Chrinomus* larva at 40–42°C (table 3).

The species diversity in both the thermal springs declined with the rise of temperature, only the procaryotic forms (blue-green algae and bacteria) were encountered at very high temperatures. The blue-greens are unicellular, non nitrogen fixing forms referable to the genus *Synechococcus* (Brock 1967). Relationship between temperature and biota is well reflected in these thermal springs. The diversity in forms increases with the fall of temperature. In other words it could be said that with the rise of temperature the significance of the population structure becomes progressively simpler and from evolutionary point of view it could be inferred that microbes found at higher temperatures are optimally adapted to this condition nearly identical to the extremes.

Acknowledgements

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Regulation of Nitrogenase Activity in *Anabaena variabilis*

P A CHANDRASEKKARAN and G S VENKATARAMAN
Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110012

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Nitrogenase activity (acetylene reduction) in *Anabaena variabilis* was examined with respect to the effects of exogenous nitrogen, amino acids, antibiotics, inhibitors of protein synthesis, analogues of glutamine and chelating agent, EDTA. The effects of NO_3^- and NH_4^+ on preformed nitrogenase were quite different in that the inhibitory effect of NO_3—N was much less than that of NH_4—N. The inhibitory effect was reversible, with the removal of NO_3^- and NH_4^+ from the medium. Except alanine, all other amino acids examined as well as methionine sulphoximine, azaserine and carbamyl phosphate inhibited nitrogenase activity to varying degrees. Inhibition of nitrogenase activity by rifampicin, streptomycin and chloramphenicol suggests a possible involvement of transcription of DNA as well as translation of mRNA. Addition of EDTA strongly inhibited nitrogenase activity and this inhibition was not reversible with Ca^{2+} or Mg^{2+}.

Key Words: Nitrogenase, Acetylene reduction, Methionine sulphoximine, EDTA, *Anabaena variabilis*

Introduction

The ability to convert atmospheric nitrogen to ammonia is restricted to certain bacteria and blue-green algae and this process is regulated both at the level of biosynthesis and catalytic activity of nitrogenase (Thomas & Tuli 1980). This communication deals with the regulation of nitrogenase activity in the heterocystous blue-green alga, *Anabaena variabilis* by exogenous nitrate- and ammonium-nitrogen, antibiotics, amino acids, analogues of glutamine and the chelating agent, EDTA.

Material and Methods

Culture of the Alga

Axenic cultures of *Anabaena variabilis* were obtained from the Culture Collection of Microalgae, Division of Microbiology, Indian Agricultural Research Institute, New Delhi. The alga was grown in 1- or 4-litre flasks, illuminated with cool fluorescence tubes at $30 \pm 1°C$. The basal medium used was nitrogen-free BG 11 (Stanier et al. 1971). Growth was monitored in terms of chlorophyll *a* content. For pigment determination,

samples were harvested by centrifugation for 5 min, extracted with 90% (v/v) methanol and quantitated at 650 nm and 665 nm (MacKinney 1941). In experiments to determine the immediate effect of exogenous NO_3^- and NH_4^+ on nitrogenase activity, 1 mM KNO_3 or NH_4Cl was added and the medium was buffered at *p*H 7.2±7.5. When exogenous substances were to be removed, the algal cells were centrifuged, washed repeatedly with nitrogen-free BG 11 medium and resuspended in BG 11 nitrogen-free medium, taking care of the initial volume of the cultures.

Besides NO_3^- and NH_4^+, other substances which were examined for their effect on nitrogenase activity were: 0.1 mg/ml rifampicin (Sigma Chemical Co., USA), streptomycin (Alembic Chemicals Works, Baroda) and chloramphenicol (Mac Lab., Bombay); 2 mM alanine, arginine, aspartate, asparagine, glutamate, glycine (Loba-Chemie Indo-Austranal Co., Bombay), glutamine (Koch-Light Lab., England); 2 mM L-methionine-DL-sulphoximine (MSX; Sigma Chemical Co., USA); 2 mM azaserine (Sigma Chemical Co., USA); 0.5 mM carbamyl phosphate (Sigma Chemical Co., USA) and 1 mM EDTA (BDH). All the solutions were properly neutralized.

Acetylene Reduction Assay

Nitrogenase activity was assayed by whole cell reduction of acetylene to ethylene (ARA) and expressed as μ mol C_2H_4 mg Chl^{-1} hr^{-1}. Acetylene equal to 10% of the total volume was injected and vials were incubated for 90 min at 30°C under 2000 lux. The reaction was terminated by injecting 0.1 ml TCA (50%) and the gas phase was assayed for ethylene, using a Neucon Model GLC 5500 with flame ionization detector and a 2 meter long Porapak R stainless steel column. The column temperature was maintained at 100°C and nitrogen gas at a flow rate of about 35 ml per min served as a carrier gas.

Results

Effect of Nitrate- and Ammonium-Nitrogen on Nitrogenase Activity

Although NO_3^- and NH_4^+ affected the nitrogenase activity in *Anabaena variabilis*, the two sources of nitrogen differed significantly with respect to their effect. There was an immediate linear decline in the nitrogenase activity upon the addition of 1 mM NH_4Cl to dinitrogen cultures (figure 1). After 8 hrs, nitrogenase activity appeared to stabilize at 20 to 35% of the rate of dinitrogen grown cultures and thereafter declined only slowly. Addition of 1 mM KNO_3, however, had no inhibitory effect on nitrogenase up to 8 hr, after which the activity declined by 18% at the end of 24 hr. With the removal of NH_4Cl and KNO_3 from the medium, the nitrogenase activity returned within

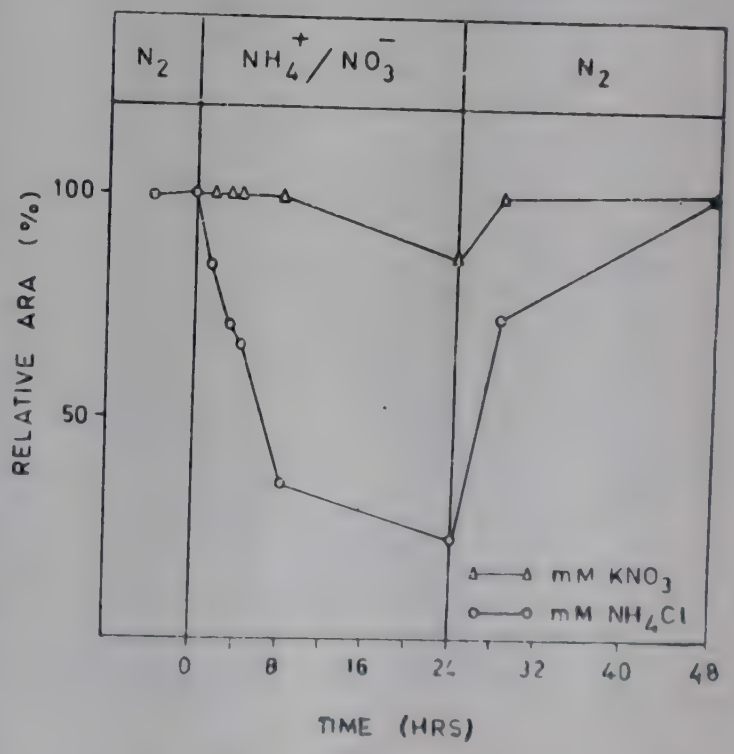

Figure 1 Effect of added nitrate and ammonium to dinitrogen growing culture of *Anabaena variabilis* on the activity of nitrogenase (acetylene reduction) as a function of time after the addition. Each point is the mean of three to five experiments. Control rate of acetylene reduction was 6.19 μmol mg Chlorophyll^{-1} hr^{-1}

24 hr to the level of the control cultures lacking nitrogen (figure 1).

Effect of Antibiotics on Nitrogenase Activity

In presence of 0.1 mg/ml rifampicin, nitrogenase activity decreased to 33% of the control within 2 hr and at the end of 20 hr, the activity was only 12% (figure 2). Streptomycin (0.1 mg/ml) also inhibited the nitrogenase activity, though the degree of inihibition was not as severe as that of rifampicin. On the other hand, chloramphenicol (0.1 mg/ml) had no effect up to 6 hr after addition, after which there was a sharp reduction in the activity reaching 22% of the control in 20 hr. With the removal of the antibiotics from the medium, nitrogenase activity increased rapidly (figure 2).

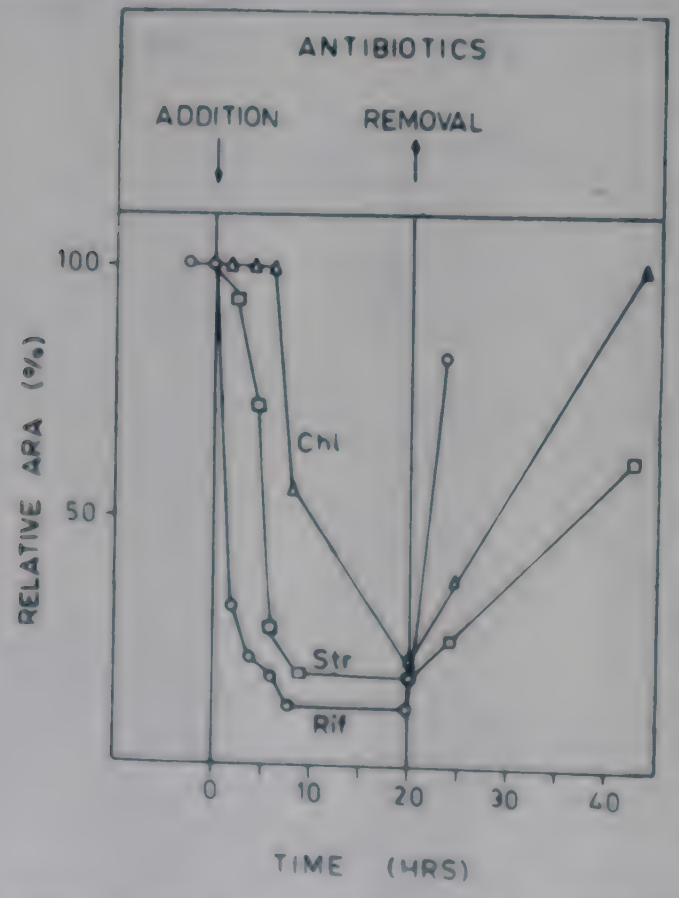

Figure 2 Effect of added antibiotics (0.1 mg/ml) to dinitrogen growing culture of *Anabaena variabilis* on the activity of nitrogenase (acetylene reduction) as a function of time. Control rate of acetylene reduction was 6.34 μmol mg chlorophyll^{-1} hr^{-1} (Rif, rifampicin; Str, streptomycin; Chl, chloramphenicol)

Table 1 *Effect of added amino acids (2 mM) on the nitrogenase activity (acetylene reduction) of* Anabaena variabilis (*figures in parantheses show the relative activity*) (*Control* 4.01 μmol C_2H_4 mg Chl^{-1} hr^{-1})

Treatment	μmol C_2H_4 mg Chl^{-1} hr^{-1}			
	2 hr	4 hr	6 hr	8 hr
Alanine	4.01 (100)	4.01 (100)	4.01 (100)	4.01 (100)
Arginine	1.32 (33)	0.75 (19)	0.4 (10)	0.4 (10)
Aspartate	0.56 (14)	0.4 (10)	0.31 (8)	0.31 (8)
Asparagine	1.76 (44)	0.75 (19)	0.4 (10)	0.4 (10)
Glutamate	4.02 (100)	3.75 (94)	0.94 (23)	0.45 (11)
Glutamine	4.01 (100)	2.08 (52)	0.45 (11)	0.25 (6)
Glycine	4.01 (100)	4.00 (100)	2.96 (74)	0.99 (25)

Effect of Amino Acids on Nitrogenase Activity

Amino acids differed significantly with respect to their effects on nitrogenase activity (table 1). Addition of 2 mM alanine had no effect up to 8 hr. Arginine and aspartate drastically reduced the activity within 2 hr of addition, the reduction being 67% in arginine and 86% in aspartate. At the end of 8 hr, the activity in presence of these two amino acids was only 8–10% of that in the control. Glycine had no effect up to 4 hrs, after which the activity declined to 25% of the control at 8 hr. Addition of asparagine resulted in a rapid decrease (44%) in the activity within 2 hr, which was further reduced to 10% at 8 hr. Glutamine and glutamate, products of NH_4^+ assimilation, also showed a repressive effect after 2 hrs of addition. At 4 hrs after addition of glutamine, the reduction in the activity was 48%, while with glutamate it was only 6%. However, at 8 hrs, only 6–11% of the rate of the control was observed in presence of both glutamine and glutamate.

Effect of Methionine Sulphoximine (MSX), Azaserine and Carbamyl Phosphate on Nitrogenase Activity

Addition of 2 mM methione sulphoximine (MSX), an inhibitor of glutamine synthetase, did not have any effect up to 2 hrs, but at the end of 8 hrs, nitrogenase activity sharply declined to about 8% of that of the control (figure 3). Similar trend was also observed with 1 mM MSX. This is rather interesting since in almost all the systems tested so far, MSX enhances nitrogenase activity. 2 mM azaserine, an analogue of glutamine reduced the nitrogenase activity by about 55% within 2 hrs of addition. At the end of the 8th hr, the relative activity was only 6% of that in the control (figure 3). Addition of 0.5 mM carbamyl phosphate also resulted in a drastic reduction in the nitrogenase activity.

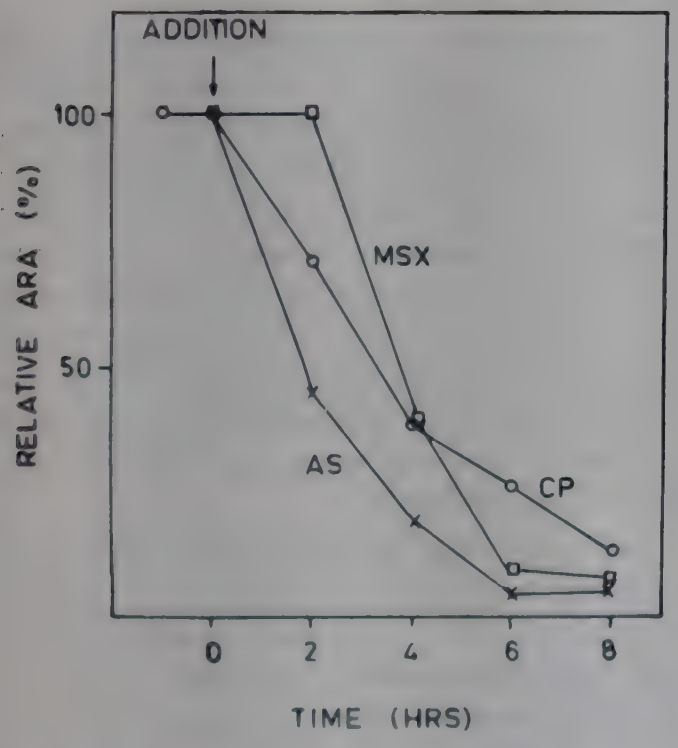

Figure 3 Effect of added methionine sulphoximine (2 mM) (MSX), azaserine (2mM) (AS) and carbamyl phosphate (0.5mM) (CP) to dinitrogen-growing culture of *Anabaena variabilis* on the activity of nitrogenase (acetylene reduction) as a function of time. Control rate of acetylene reduction was 6.2 μmol mg chlorophyll^{-1} hr^{-1}

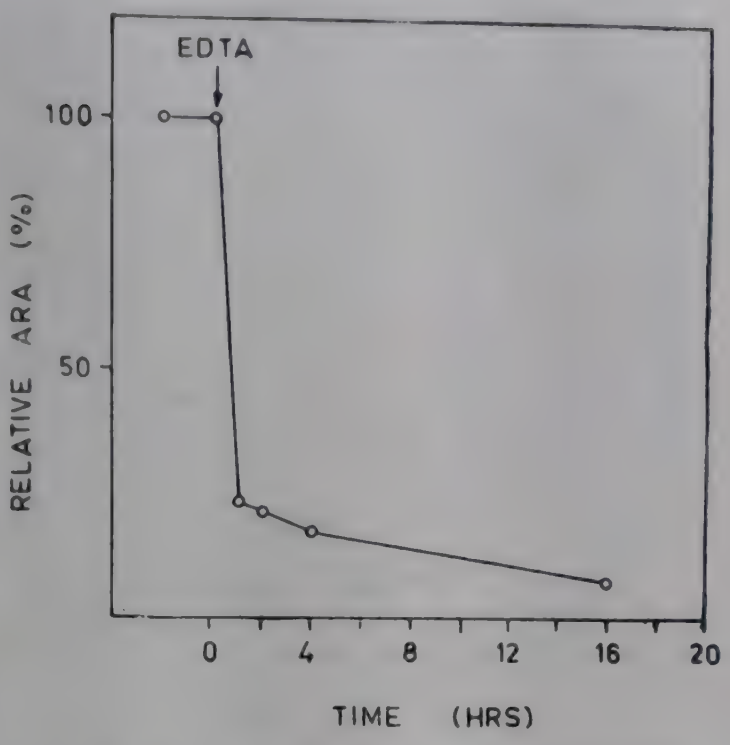

Figure 4 Effect of added EDTA (1mM) to dinitrogen-growing culture of *Anabaena variabilis* on the activity of nitrogenase (acetylene reduction) as a function of time. Control rate of acetylene reduction was 5.22 μmol mg chlorophyll^{-1} hr^{-1}

Effect of Ethylene Diamine Tetra-acetic Acid (EDTA) on Nitrogenase Activity

There was an immediate linear decline in the nitrogenase activity upon the addition of 1mM EDTA, until very little activity could be detected after 16 hr (figure 4). However, addition of EDTA had no effect on photosynthetic oxygen evolution. To examine the ability of $CaCl_2$ and $MgCl_2$ to reverse the EDTA-induced inhibition, 5mM $CaCl_2$ or $MgCl_2$ was added to the cultures 1 hr after the addition of EDTA. Figure 5 shows that there was no reversal of the inhibition caused by EDTA.

Discussion

The repression of nitrogen fixation by ammonium has been confirmed on a variety of blue-green algae (Fogg et al. 1973, Haselkorn 1978, Meeks et al. 1983). The only reported exception is the marine *Anabaena* CA, in which nitrate and not ammonium represses

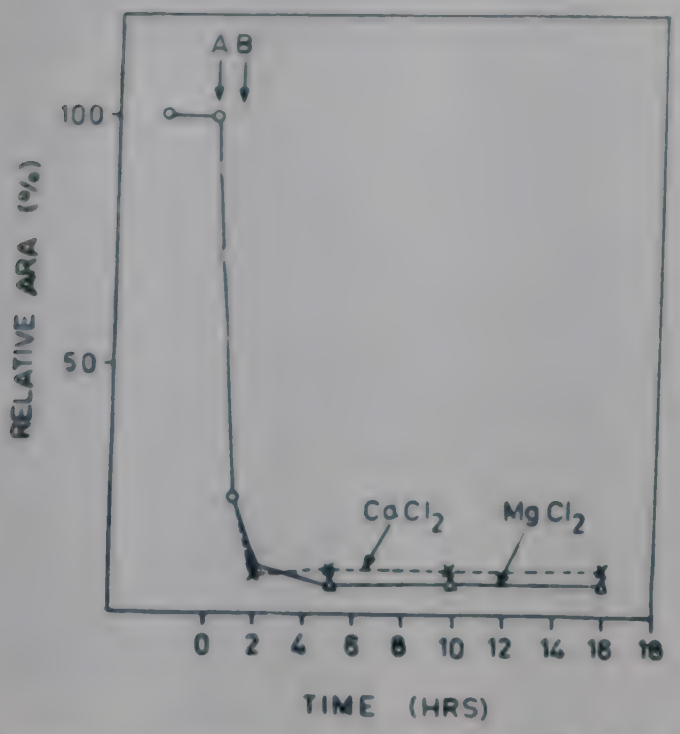

Figure 5 Effect of added $CaCl_2$ and $MgCl_2$ (5mM) (B) on the EDTA-induced inhibition (A) of nitrogenase activity (acetylene reduction) in *Anabaena variabilis*. Control rate of acetylene reduction was 5.22 μmol mg chlorophyll^{-1} hr^{-1}

heterocyst production and nitrogen fixation (Bottomley et al. 1979). The time course decline in nitrogenase activity in *Anabaena variabilis* with NH_4^+ (figure 1) was fairly similar to that observed in other blue-green algal strains (Adams & Carr 1981, Stewart & Rowell 1975, Meeks et al. 1983). Besides repression, NH_4^+ may cause inactivation of the existing nitrogenase as in *Azotobacter* (Shah et al. 1972), photosynthetic bacteria (Nielson & Nordlund 1975, Zumft & Castillo 1978) and *Klebsiello* (Tubb & Postgate 1973). While addition of NH_4^+ brought about an immediate linear decline in the nitrogenase activity, the effect of NO_3^- was quite different, in the sense that there was no inhibition of the activity up to 8 hr after addition of NO_3^-, after which the activity declined by only 18% (figure 1). When both NO_3^- and NH_4^+ were removed from the medium, nitrogenase activity returned to its original level within 24 hr. Much of the loss of the activity may be probably due to a simple dilution out of the enzyme with growth, but it is possible from the rapid drop-off in activity that there is also a rapid turnover of nitrogenase protein without resynthesis (Stewart et al. 1975). It is clear that the effects of NO_3^- and NH_4^+ on preformed nitrogenase are quite different in that the inhibitory effect of NO_3—N on nitrogenase synthesis is much less than that of NH_4—N.

Besides NH_4^+, the overall mechanism of nitrogenase regulation seems to involve amino acid levels (Thomas 1978, David & Thomas 1980). Except alanine, all other amino acids examined inhibited nitrogenase activity in *Anabaena variabilis* to varying degrees (table 1). Low levels of exogenous glutamate inhibit nitrogenase activity in *Anabaena* L-31, the inhibition being, however, alleviated by asparate (Thomas 1978, David & Thomas 1980).

The inhibition of nitrogenase activity in *Anabaena variabilis* by rifampicin, streptomycin and chloramphenicol (figure 2) suggests that transcription of DNA as well as translation of mRNA might be involved. Other compounds that inhibited nitrogenase activity were 0.5 mM carbamyl phosphate and 2mM azaserine, an analogue of glutamine which inhibits glutamate synthase and causes an accumulation of glutamine and 2 mM methionine sulphoximine, an inhibitor of glutamine synthetase.

The inhibition of nitrogenase activity in *Anabaena variabilis* by EDTA is rather interesting. EDTA is a polar molecule which does not usually enter the cells. It must, therefore, exert its effect through the decrease in the external concentration of free divalent ions. This could lead to the diffusion of these cations out of the cells, lowering the intracellular concentrations of such ions as Ca^{2+} and Mg^{2+} and this might in turn inhibit acetylene reduction. The heterocystous algae

are normally insensitive to inhibition by EDTA, because their nitrogenase activity under aerobic conditions is located in the heterocysts, whose thick walls would be less likely to permit ion diffusion than would the vegetative cells. This would imply that the heterocystous forms like the present one upon which EDTA has an effect may have a vegetative cell nitrogenase, even though they are grown aerobically. The occurrence of nitrogenase in vegetative cells of heterocystous forms when grown under microaerophilic conditions is known (Stewart 1974). The role of EDTA in the overall ATP generation also deserves to be looked into. The response of blue-greens to EDTA varies from species to species. For example, EDTA completely inhibits photosynthetic oxygen evolution and carbon dioxide fixation in *Anabaena cylindrica*, consequently affecting its acetylene reduction activity. On the other hand, it has no effect on photosynthetic oxygen evolution in *Gloeocapsa Anabaena flos-aquae*, *Aphanizomenon flos-aquae* (Gallon 1978) and in the present form, but inhibits acetylene reduction. Thus, in these forms the two processes do not seem to be related. Caution must, therefore, be exercised while extrapolating the result from one alga to another. The present strain of *Anabaena variabilis* resembles *Aphanizomenon flos-aquae* in that the inhibition by EDTA is not reversible either by $CaCl_2$ or $MgCl_2$, which, at least in part, reverses the inhibitory effect of EDTA in *Anabaena cylindrica* and *Anabaena flos-aquae* (Gallon 1978).

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Leaching of Sulfide Ores by *Thiobacillus ferrooxidans*

SUBHANSU S ROY MAHAPATRA, PAMPA ROY, AJIT K MISHRA* and DEBABRATA CHANDRA†

**Department of Microbiology, Bose Institute, Calcutta 700 009*
†Indian School of Mines, Dhanbad, Bihar

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Leaching of various sulfide ores using strains of *T. ferrooxidans* was undertaken. Effective oxidation occurred under suitable physico-chemical conditions viz., *p*H, temp, pulp density, adaptation, nutrition etc. Inspite of the experimental conditions being the same the optimum pulp densities for pyrite and pyrrhotite oxidation were different. Pyrrhotite treated with 2(N) H_2SO_4 as well as cells enriched in pyrrhotite gave maximum oxidation. Investigations on the bacterial activity during leaching of mixed metal sulfide ores are also discussed.

Key Words: *Thiobacillus*, **Sulfide ore, Polymetallic sulfide ore, Leaching**

Introduction

The microbiological leaching of valuable metals from the mineral ores using *Thiobacillus ferrooxidans*, is now attaining a feasible commercial shape (Torma & Bosecker 1982). Intensive studies indicate that the bacterium possesses great potentiality for oxidation of sulfide minerals and in some cases the reduced metal part itself is used as the substrate (Kelly et al. 1979, Lundgren & Silver 1980). Sulfide minerals are mostly associated with iron, which can be oxidised by the bacterium for its energy yielding process (Torma 1977). However, the iron should be available here in its reduced form. In general, the insoluble metal sulfides come out as soluble metal sulfates into solution of acidified leach media. This process is enhanced by the bacterially regenerated acid ferric sulfate which plays a vital role by its oxidising action (Brierley 1978). The involvement of microbes during leaching of sulfide minerals has been well studied (Duncan et al. 1967, Bruynesteyn & Duncan 1971, Atkins 1978) and has also been reviewed (Torma 1977, Lundgren & Silver 1980). Still, quantitative data, on the parameters of such techniques on bioleaching of low grade ores, sulfide minerals contaminated with toxic elements, complex metal sulfides or polymetallic sulfide ores, is very much limited. The objective of this study was to investigate quantitatively the parameters that may affect the leaching of such mineral ores of Indian origin by *T. ferrooxidans*, as these bacteria have their natural inhabitance in most of such environments (Mishra et al. 1983).

Materials and Methods

For oxidation studies, the samples were collected from different parts of India and ground to particle size $<63\mu$. The major mineralogical and chemical constituents of these samples are given in table 1.

T. ferrooxidans culture (NCIB-8455, India and some indigenous isolates of *T. ferroxidans* from Rajpura, Dariba and Deri, Rajasthan) were used in these studies. The cultures were routinely maintained in the modified 9k medium (Mishra et al. 1983). Instead of ferrous sulfate suitable amount of the sulfide samples were added to this medium (*p*H 2.0) for leaching experiments.

The cell suspensions (5×10^9 cells/ml) were prepared from each substrate adapted culture by harvesting on membrane filter (0.45μm, pore size) and resuspending the same in 0.01 (N) H_2SO_4. 1.0 ml of this cell suspension was inoculated in 100 ml medium containing requisite amount of the sample and incubated at 28°C under shaking condition (150rpm). Suspensions from the growing culture were taken intermittently to estimate water-soluble metals according to the standard procedures (Snell & Snell 1949). The amount of metals or toxic elements initially present in the mineral ores were also determined in a similar manner. Total sulfur in each sample was estimated as described earlier (Roy & Mishra 1981). Strain NCIB-8455 was used for bacterial Pyrite and Pyrrhotite oxidation and RDM-7 was used for oxidation of mixed metal sulfide ores.

Results

Bacterial Pyrite Oxidation

Effect of initial pH : The leach curves (figure 1a & b) show that, at 2.5% pulp density the rate of oxidation of the sulfide sample (No. 1), containing major pyrite minerals (43.05% pyritic iron), was maximum at *p*H 2.5 (14.2 mg iron/1/hr); whereas, maximum oxidation occurred at *p*H 2.0 (70%). At *p*H below 1.5, the oxidation was negligible. The iron was leached from pyrite exponentially, continuing up to 15 days. The optimum *p*H was found between 2.0 and 2.5 by considering leach rate and percent oxidation. At *p*H 2.0 where maximum oxidation occurred, the mercury concentration in the leached solution was nearly 1.5 mg/l.

Effect of pulp density: There was no lag period for pyrite oxidation as studied upto 10% pulp density. The iron release was very slow for the initial 6 days (figure 2a).

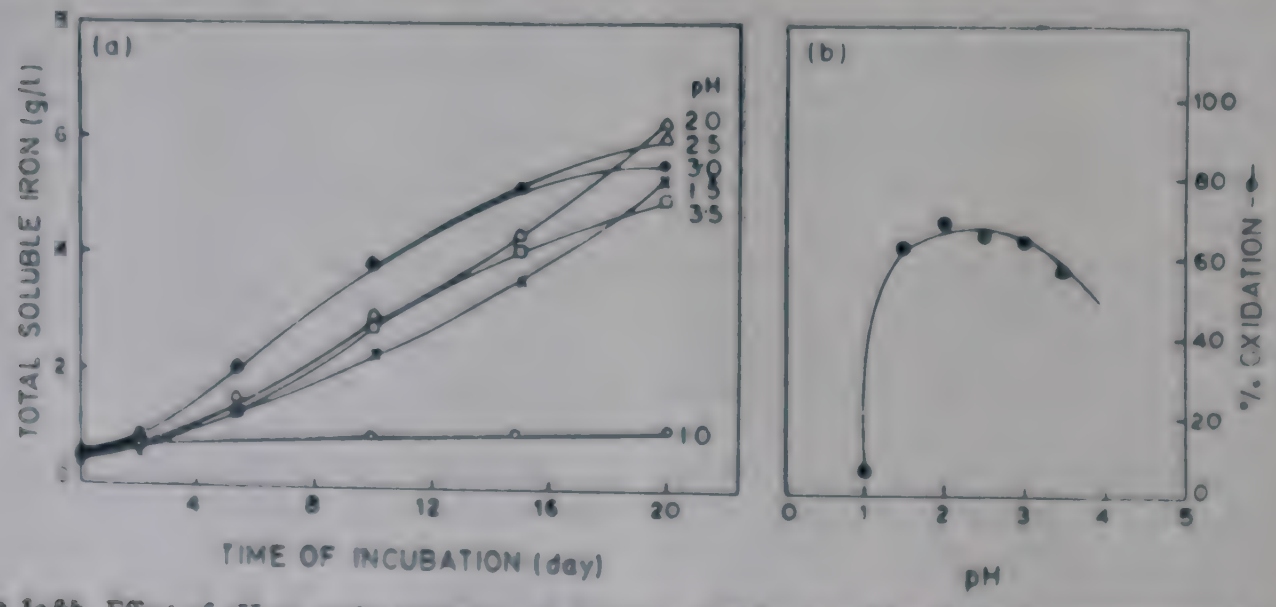

Figure 1a&b Effect of *p*H on pyrite oxidation of Rajpura sulfide ore (No. 1) by *T. ferrooxidans* at 2 5 % pulp density

Table 1 *Major constituents of sulfide-bearing ores and minerals*

Sample No.	Sample	Location	Mineralogical	Chemical (% w/w)							
				Fe	Cu	Pb	Zn	Ag	Hg	Cd	S
1	Sulfide ore	Rajpura, Rajasthan, India	Pyrite, Spharelite, Galena, Quartz.	43·05	0·05	2·50	1·57	—	0·01	—	50·38
2	,,	Saladipura, Rajasthan, India	Pyrrhotite, Pyrite Sidarite.	46·50	0·09	1·09	—	—	—	0·02	31·46
3	,,	Deri, Rajasthan, India	Pyrite, Spharelite Galena, Chalcopyrite Quartz.	36·54	1·14	6·87	9·12	0·006	—	—	45·38
4	Copper ore tailings	Mosaboni, Bihar, India	Chalcopyrite, Sidarite Quartz.	0·10	0·05	—	—	—	—	—	0·18
5	Copper ore	Malanjkhand, Madhya Pradesh, India	Chalcopyrite, Covellite, Quartz.	4·00	5·00	1·05	0·65	—	—	0·01	7·65
6	,,	Rangpo, Sikkim, India	Chalcopyrite, Covellite, Galena, Quartz.	5·80	1·08	2·45	0·31	0·001	—	0·005	8·56
7	Zinc ore tailings	Zawar, Rajasthan, India	Spharelite, Pyrite, Pyrrhotite, Sidarite Quartz.	2·47	—	—	3·60	—	—	—	3·29
8	Sulfide ore	Ambamata, Gujarat, India	Pyrite, Pyrrhotite, Chalcocite, Quartz.	41·20	1·31	1·50	1·05	—	0·002	—	48·19
9	,,	Dariba, Rajasthan, India	Pyrite, Covellite, Chalcocite, Galena Quartz.	2·56	2·35	0·86	0·10	0·02	—	—	4·69
10	,,	Bandalamoth, Andhra Pradesh, India	Galena, Pyrite, Sidarite, Quartz.	3·85	—	9·66	—	—	—	0·01	8·44

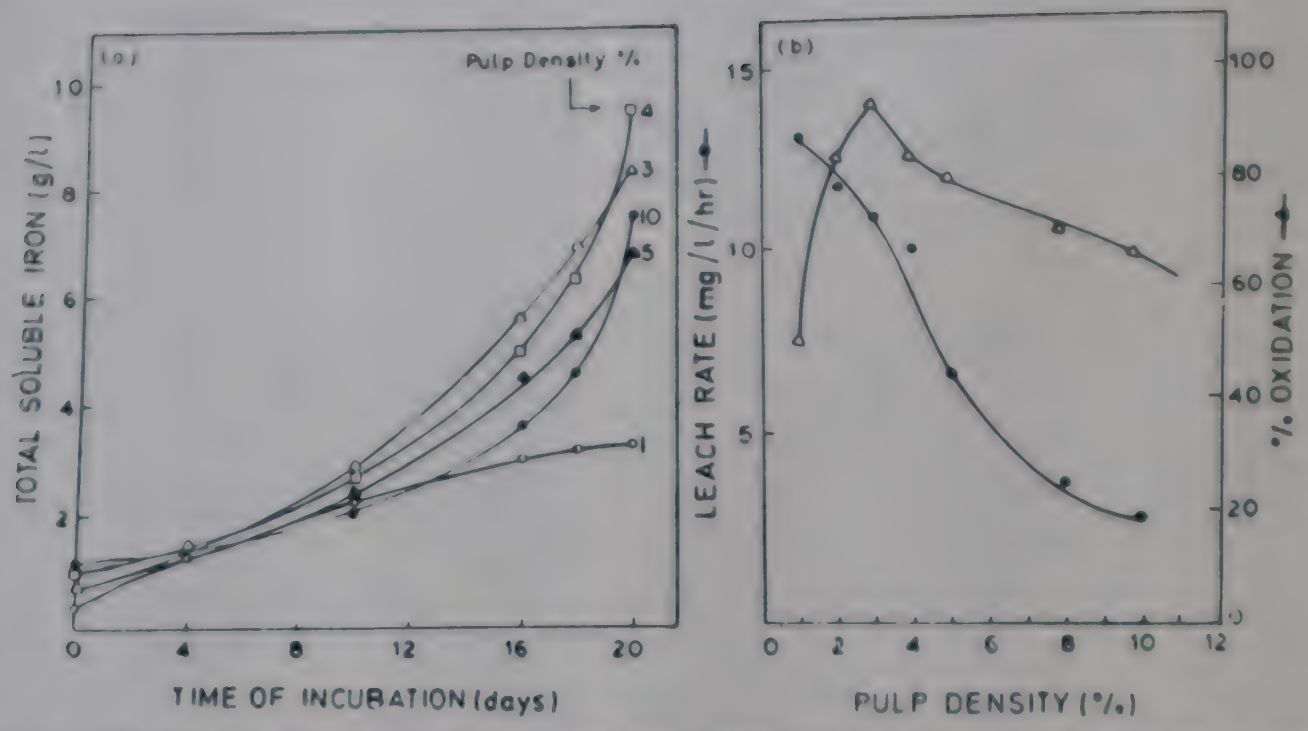

Figure 2 a&b Effect of pulp density on pyrite oxidation of Rajpura sulfide ore (No. 1) by *T. ferrooxidans*

The rate of leaching increased with increasing ore concentrations up to 3% pulp density (14 mg iron/l/hr). Above this concentration the rate gradually decreased. The optimum pulp density was around 1% to 3% when both leach rate and percent oxidation were considered (figure 2b). This sample (No.1) containing high concentrations of mercury along with the toxic elements as lead and zinc did not show any deleterious effect on pyrite oxidation by the bacterium. At higher pulp densities, i.e. above 3% (w/v), the mercury concentration in the leached solution did not exceed above 2.0 mg/l.

Bacterial Pyrrhotite Oxidation

Effect of pulp density: With another sample (No. 2) containing major pyrrhotite minerals (46.5% total iron), it was observed that, steady leach rate occurred below 5% pulp density (27 mg iron/l/hr) This sample on the other hand showed maximum oxidation (92.2%) at 1% pulp density. The rate of exponential oxidation increased steadily up to about 18 days. The optimum pulp density in case of pyrrhotite sample was between 1% and 5% by considering both oxidation and leaching profile (figure 3 a & b). Associated toxic metals like lead and cadmium did not restrict oxidation in any case.

Effect of adaptation on various substrates: Pyrrhotite oxidation of this sample (No. 2) was followed with different substrate adapted cells. Pyrrhotite grown cells showed highest metal release which was comparable to that of pyrite and iron grown cells. Thiosulfate grown cells gave least soluble iron extraction, although the activity of sulfur or chalcopyrite grown cells were substantially better (figure 4).

Pyrrhotite oxidation under various conditions: The sample (No. 2) showed various iron leach profile under different conditions (figure 5). At higher temperature, the oxidation decreased. Unsterilised sample in only acidified water (*p*H 2.0) showed significant oxidation, which was further increased by the addition of nutrients. Dilute H_2SO_4 (2N) pretreated sample gave maximum metal release (76%), whereas, water washed pyrrhotite showed nearly similar oxidation

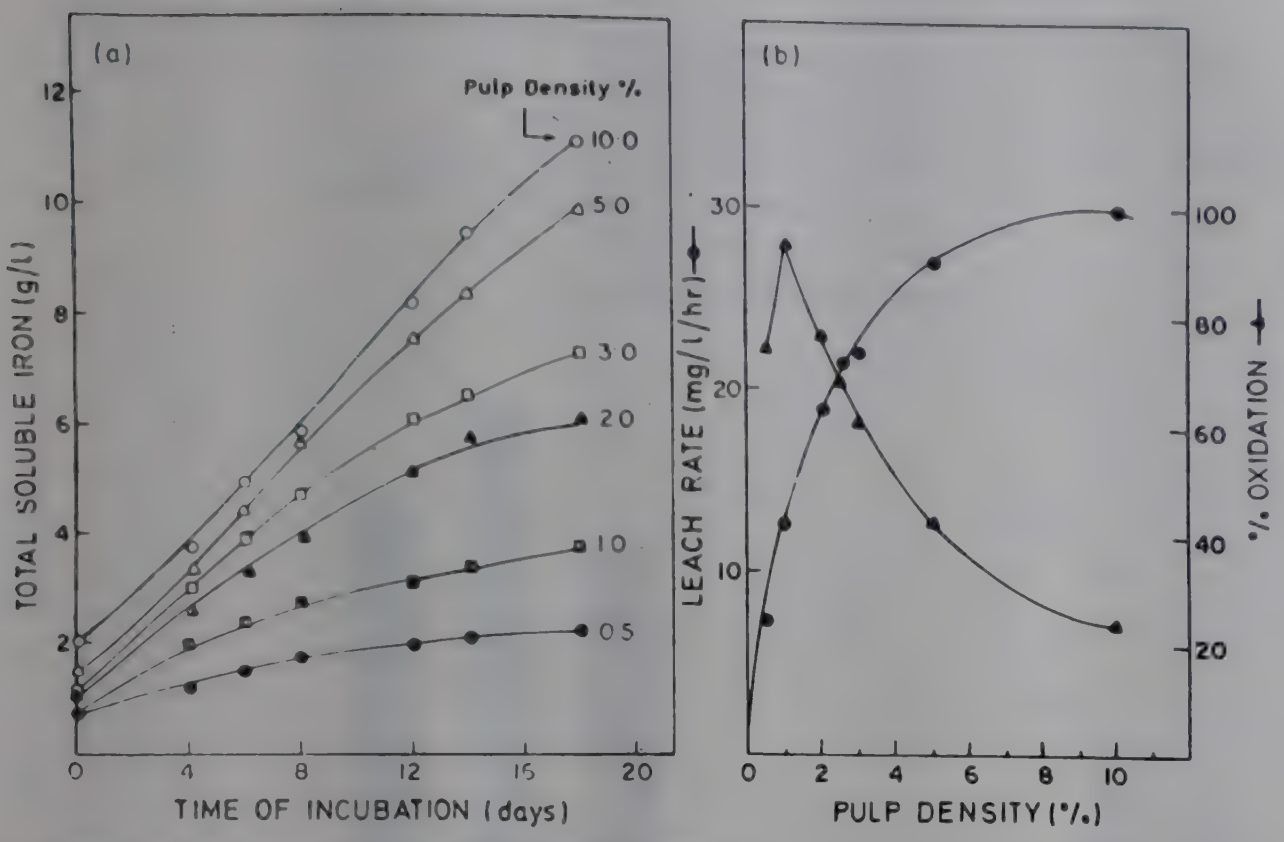

Figure 3 Effect of pulp density on pyrrhotite oxidation of Saladipura sulfide ore (No. 2) by *T. ferrooxidans*

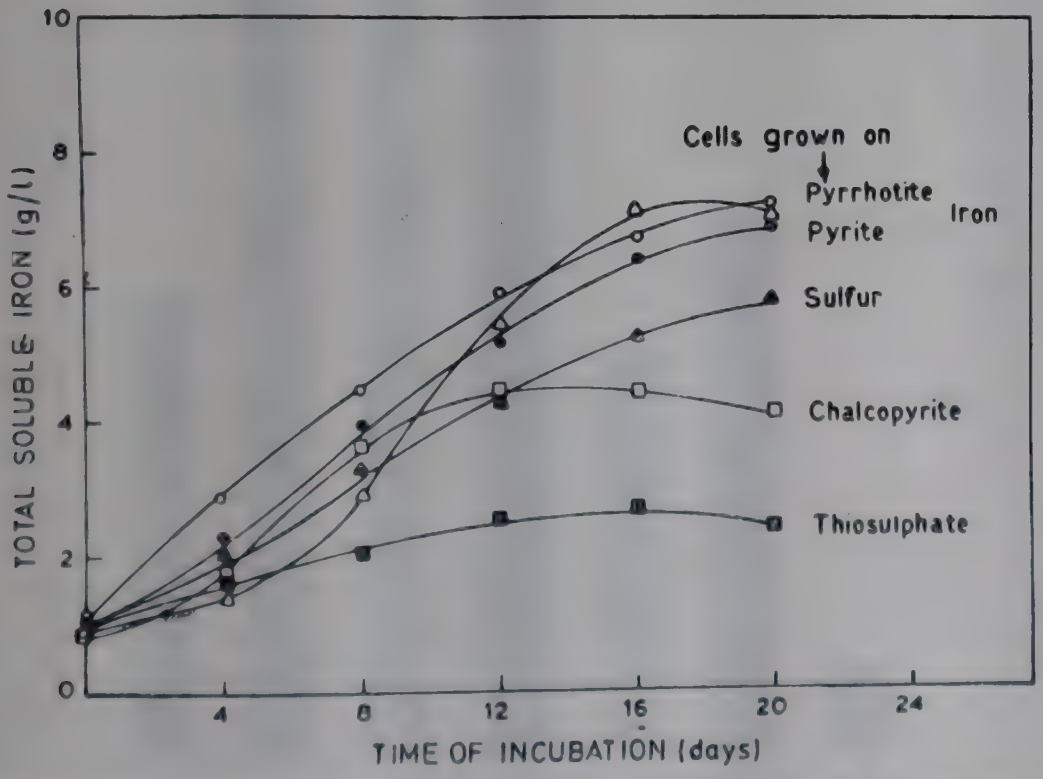

Figure 4 Effect of different substrate adapted cells of *T. ferrooxidans* on pyrrhotite oxidation of Saladipura sulfide ore (No. 2) at 3% pulp density and at *p*H 2·0

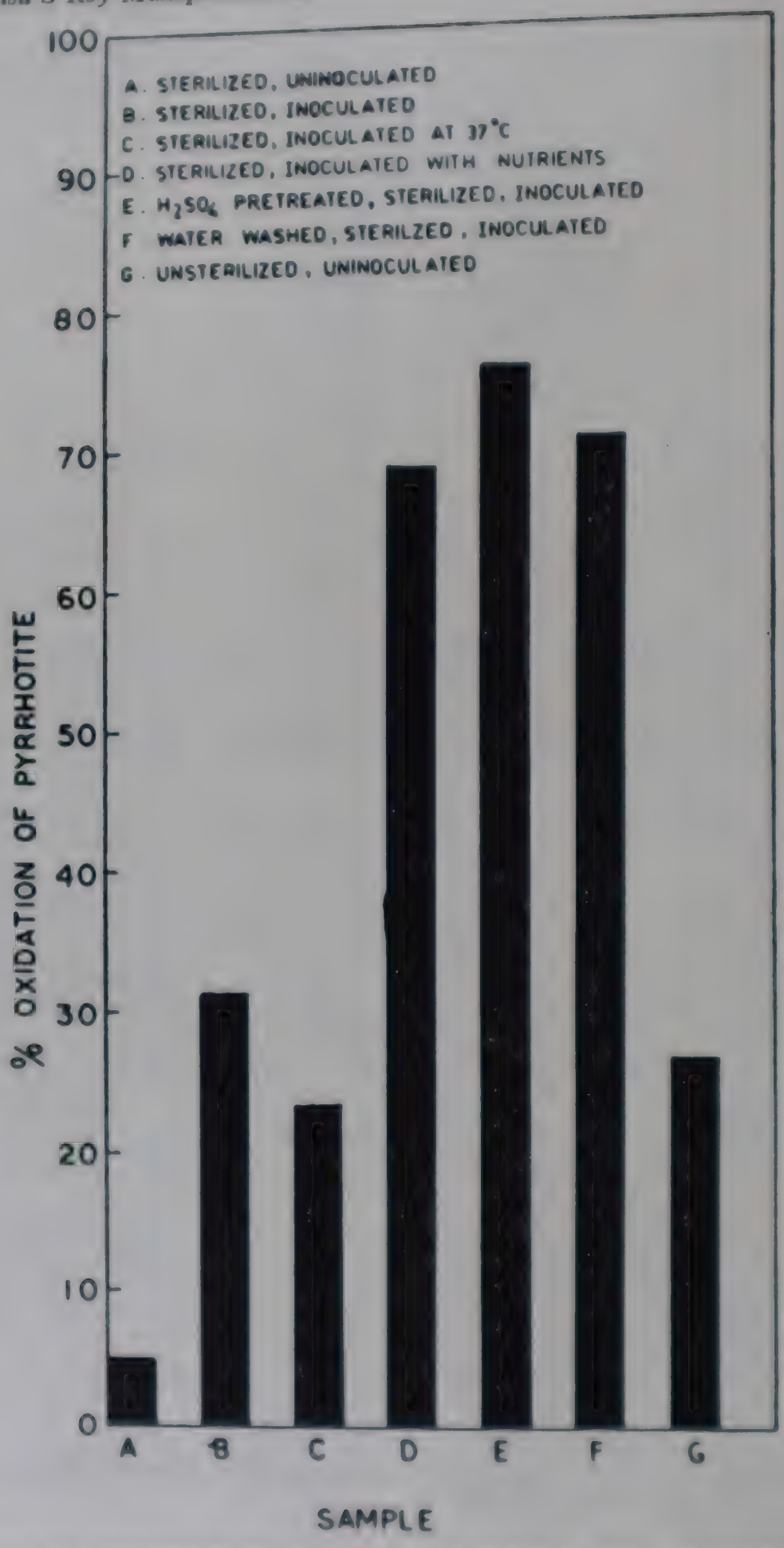

Figure 5 Oxidation of Saladipura pyrrhotite (No. 2) after 20 days under various conditions by *T. ferrooxidans* at 3% pulp density in acidified water (*p*H 2·0) and at 28°C

compared to the sample without any pretreatment, where 70% oxidation had occurred.

Bacterial Leaching of Polymetallic Sulfide Ores

The leaching of an indigenous isolated bacterial strain RDM-7 (source — Rajpura, Dariba Mine ore; Sample—7) in the presence of different mixed metal sulfide ores is shown in figure 6. The strain was quite effective in solubilising such complex ores. Studies on leaching of a lead, zinc and silver rich pyrite sample (No. 3) containing 33.0% pyritic iron and 1.14% copper in shake flasks at 2.5% ore pulp density were carried out. After 20 days about 57.8% iron came into solution, whereas more than 95% copper was solubilised (figure 6a). The final leach solution was estimated for the concentration of soluble toxic elements which accounted for 78 mg/l for lead, 159 mg/l for zinc respectively.

The strain was efficient in leaching 92% copper in 30 days from a copper ore tailings sample (No. 4) containing as low as 0.05% copper (figure 6b). In most of the cases this sample (5% w/v) was supplemented with a low grade pyrite (5% w/v) having 12.25% sulfur. The metal release was reduced to nearly 50%, without pyrite addition. Increase in tailings concentrations reduced the copper leach profile. In the sterile control, nearly 6% of the copper was solubilised in 30 days.

At 2.5% pulp density, 73.3% copper was solubilised from one copper ore sample (No. 5) containing 5% copper and 82% from another silver contaminated copper ore sample (No. 6) containing 1.08% copper. These samples were associated with another toxic element — lead, of which nearly 75 mg/l was in soluble form. In both the cases, the iron leach curves were proportional to exponential copper leaching (figure 6 c & d).

Another zinc ore tailings (No. 7) contained 3.6% zinc, 40% of which was solubilised in 20 days by the ore adapted strain. The sample during the same period of leaching showed nearly 65.5% total iron solubilisation (figure 6e).

In 20 days, 80% zinc could be leached from a mercury containing metal sulfide ore which contained zinc as low as 1.05%. This sample (No. 8) was associated with 1.3% copper of which 87.3% was solubilised (figure 6f). The amount of lead was nearly 78 mg/l in the leached solution. Significant amount of mercury (1.0 mg/l) was also found to be solubilised. The pyritic iron content of the sample (41.2%) was reduced to about 4.8% by 20 days treatment.

Another polymetallic sulfide ore containing only 2.5% pyritic iron, showed 92% zinc solubilisation in 20 days (figure 6g). This sample (No. 9) was associated with as low as 0.1% zinc, 2.35% copper and some toxic elements like lead and silver. Nearly 80% copper came into solution during this period although the concentration of lead was not more than 50 mg/l in the leaching media.

The sulfide ore (No. 10) containing galena as a major sulfide mineral does not show effective leaching, although the final concentration of lead in the leached solution was found to be 125 mg/l. Significant amount of cadmium and zinc were solubilised in most of the cases where these were present in the ore. Silver contaminated sulfide ores did not show appreciable silver solubilisation in any case.

In most cases acidity was found to increase to a final *p*H value of 1.2 to 1.5, although in some cases the initial *p*H (2.0) increased to about 3.5 to 5.0 which had to be readjusted with 0.01 (N) H_2SO_4.

Discussion

The bacterium *T. ferrooxidans* showed maximum solubilisation of metal sulfides between *p*H 2.0 to 2.5, which is in agreement

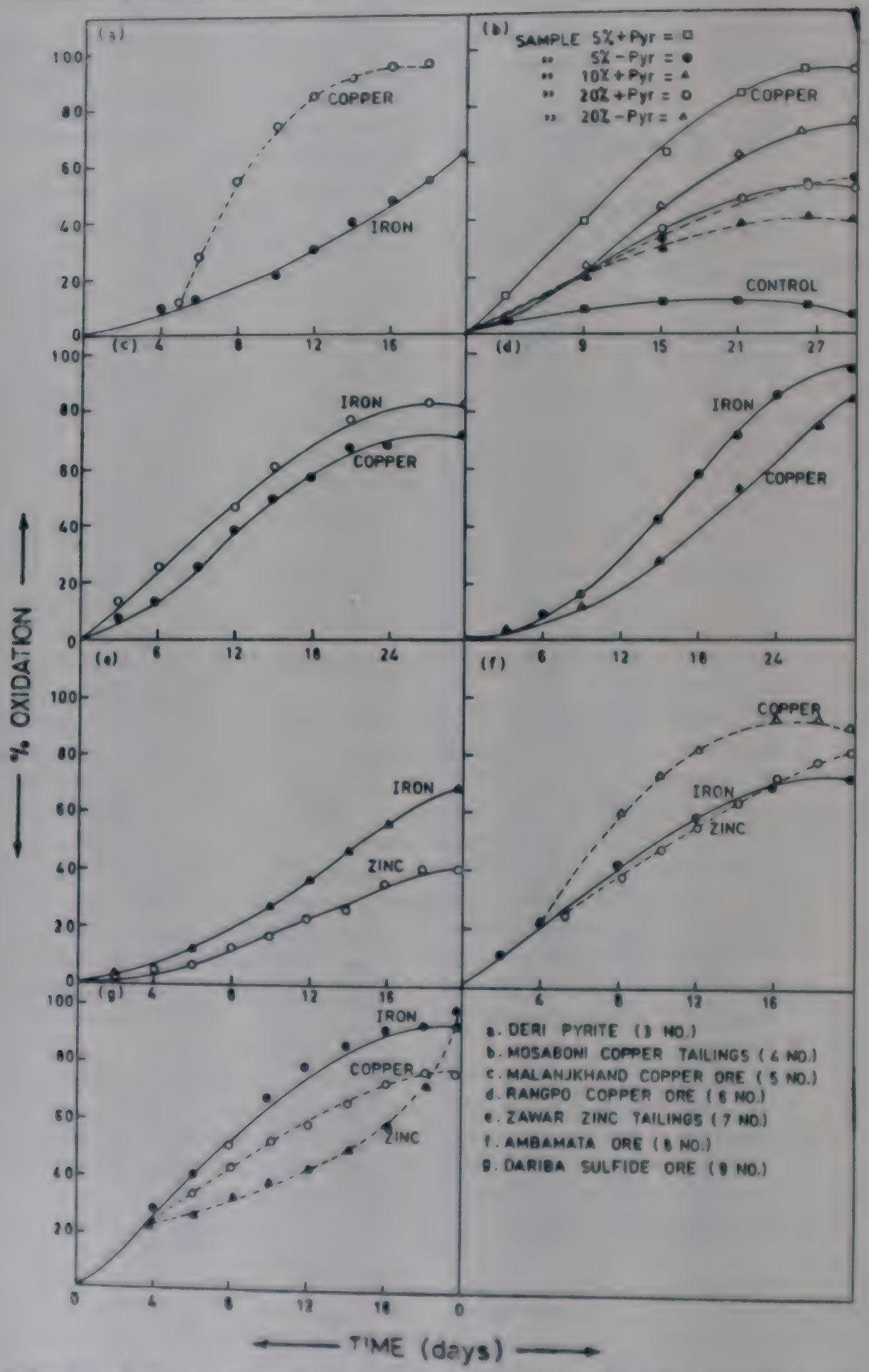

Figure 6 Leaching of some polymetallic sulfide ores by *T. ferrooxidans* at 2·5% pulp density and *p*H 2·0

with the earlier reports (Duncan et al. 1967, Atkins 1978). During this process the acidity is increased, and as such, the bacterial activity is reduced after a particular *p*H drop, which has been shown here to be below 1.5.

During pulp density experiments it was observed that, in the case of pyrite sample the optimum pulp density was around 2% (w/v), whereas for the pyrrhotite sample it was near 3% (w/v) though particle size was same in both the cases. Such difference probably arises due to the nature of crystal lattice of pyrite and pyrrhotite minerals (Krouse & McCready 1979). The bacterial activity in relation to their attachment to specific substrates may also differ due to lattice imperfections occuring during sheaving. It could also depend on the presence of some toxic impurities within the minerals (Roy Mahapatra & Mishra 1984). Probably for these reasons the favourable leach rate for pyrrhotite is up to 5% pulp density. The results clearly indicate that, during leaching of metal sulfides by *T. ferrooxidans* the optimum pulp density should be judged on the basis of its mineralogical specifications. Besides, the problem of oxygen mass transfer as well as attrition of cells with solids at higher pulp densities may also result in such reduced leach rate (Torma 1977, Lundgren & Silver 1980). The contributions of these factors on solid to liquid ratio in case of low grade ores is not valid due to the presence of associated host rocks with the sulfide minerals. Still lower pulp density (2.5%) was used in most of the cases by considering the presence of some toxic elements, and where such possibilities of bacterial growth retardation are negligible.

The bacterium *T. ferrooxidans* needs specific adaptation to a particular substrate for reproducible leaching of metal sulfides (Torma 1977). Direct contact between the cells and sulfide particles are also necessary to initiate such oxidation (Silverman 1967). During this adaptation period surface active agents may be produced to favour the process (Torma 1977). The requirement of adaptation has further been confirmed by Roy and Mishra (1981). It is not easy to explain at the present stage as to how the sulfur or iron grown cell types showed nearly similar metal release like pyrrhotite grown cells. But it is possible that some changes in the cell types might have occurred during enrichment (adaptation) in a particular substrate. Addition of essential nutrients, i.e. 0.1% ammonium sulfate and 0.05% dipotassium hydrogen phosphate favoured the leaching process which indicates that their optimum amounts were not present in the sample. The number of cells present in metal sulfides were found almost proportional to their total sulfur content, and the indigenous cells gave better metal sulfide oxidation than the standard strain (unpublished data). As such, further experiments on mixed metal sulfide ores were carried with a potent inherent strain RDM-7 instead of NCIB-8455. The presence of acidophilic iron oxidizers were confirmed in case of unsterilized pyrrhotite samples. The results indicate that the indigenous strains are potent under suitable physico-chemical conditions which may eliminate the cost of applying standard strain in metal sulfide leaching. The strain (RDM-7) showed effective solubilisation of metals from a variety of mixed metal sulfide ores contaminated with several toxic elements. The bacterium *T. ferrooxidans* is quite sensitive to some toxic metals at very low concentrations (Lundgren & Silver 1980), specially, silver, mercury, molybdenum, uranium and cadmium which need proper curing treatments during leaching operations (Norris & Kelly 1978). Better pyrrhotite oxidation obtained in sulfuric acid pretreated samples also supports this.

Our findings suggest that, any natural leaching environment is best for procuring

potent selective strain of *T. ferrooxidans*. The extent of solubilisation of metals by such developed strain from mixed metal sulfide ores is not found to be dependent on the absolute concentration of any metal initially present in the ore. Increase in medium *p*H as observed in some cases was probably due to presence of some basic materials in the sample. Silver contaminated mineral ores did not show significant amount of soluble silver, which could be due to its formation of complexes or precipitation with the medium constituents. The ore tailings sample gave better metal release in association with pyrite, which indicates that the amount of the substrate is not sufficient to promote optimum bacterial activity. Since, any metal sulfide leaching is a combination of both chemical and bacterial oxidation (Silverman 1967), the effective substrate availability should be adhered to during such operation. The data are not sufficient to explain exact mechanisms by which the bacterium survived in such sulfide ores but it is probable that, during their enrichment in individual ores some development of multiple metal tolerance could have occurred, or that the toxic metal did not exceed the critical concentration in the leached solution to inhibit the bacterial activity. Whatever may be the mechanism of metal tolerance by the bacterium, it is conceivable from these experiments that, any sulfide ore may be prone to bacterial attack after some enrichment in the said ore. As the bacterium *T. ferrooxidans* is usually present in most of the sulfide-bearing ores and minerals (Mishra et al. 1983), the selection or development of a particular group of microbes is probably quite easy under natural environment. As such, different types of mineral ores will help in selecting their own leaching environment to promote bacterial activity. This fact is reflected in our results. The leaching behaviour of different mixed metal sulfides by this bacterium also suggests that, the bacterial activity is always synonymous to iron leach profile. The concentration of other leached metals proportionately depends upon the iron leach curve and the other metals are leached only after a particular ferric ion concentration in the leached solution. Hence, the propogation of iron-oxidizer depends upon the concentration of the reduced iron and sulfur moiety which are preferred attackable substrates in case of mixed metal sulfide oxidation. Presence of toxic elements does not hamper their activity during such oxidation probably due to selection of microbes under natural environment.

From the economic point of view, the results presented here show better potential for leaching of sulfide containing ores and minerals by locally isolated microbial strains. India possesses substantial deposits of sulfur containing low grade ores and it may be worthwhile in the future to recover valuable metals as well as sulfur by application of such bioleaching process. Use of optimum conditions and development of suitable strains, as stated here, may show a way to enhance the applicability of this process.

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In vitro Culture of *Vanda* Hybrid (*Vanda* TMA × *Vanda* Miss. Joaquim) I. Studies on Protocorm Explants

V H MATHEWS and P S RAO

Plant Biotechnology Section, Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085

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Various parameters controlling the growth of orchid protocorms have been investigated. Study of different carbon sources revealed that 2% sucrose was the best source. Quantity of the medium had a direct correlation with the rate of proliferation of isolated protocorms. Slicing of protocorm into halves doubled the rate of multiplication. Auxins alone were conducive for protocorm multiplication, whereas cytokinins alone caused necrosis. Auxin and cytokinin combinations enhanced differentiation of protocorms. CM in the basal medium (+sucrose) stimulated growth at 5–20% concentrations, while higher concentrations were inhibitory. 40% CM in sucrose-less basal medium gave proliferation of protocorms similar to that observed with 10–15% CM on basal medium with sucrose. The development of the protocorm to seedling, or to undergo multiplication, was dependent on media components and size of initial explant. Conditions for induction of callus and regeneration were also standardized.

Key Words: **Protocorms**, **Regeneration**, *Vanda*, **Proliferation**

Introduction

Successful commercial application of tissue culture technique in plant propagation started with orchid genera and is now an accepted method for rapid propagation. However, a survey of literature shows that data on nutritional aspects of orchid embryos in culture is still meagre, except for a few reports (Kusumoto 1979 a, b, 1980 a, b, Stoltz 1979). Factors affecting seed germination and seedling development *in vitro* were studied using three different interspecific hybrids of *Vanda* (Mathews & Rao 1980). The present paper reports results of investigations on protocorm multiplication and development in the hybrid embryos of *Vanda* TMA × *Vanda* Miss. Joaquim.

Materials and Methods

Seeds of *Vanda* TMA from Bangkok × *Vanda* Miss. Joaquim from Port Moresby Botanical Garden were germinated aseptically. Modified basal medium (Mathews & Rao 1980) was used with hormonal supplements. Nitsch medium (1951) was used at the fina

stage of seedling growth, before transplantation. Various parameters such as the effect of different carbon sources (sucrose, maltose, fructose, glucose, mannose), various concentrations of sucrose, CM, GA, auxins (IAA, NAA, 2, 4-D, BTOA, IBA), cytokinins (Kn, BA, 2, i-P, Zeatin), quantity of liquid medium vs solid medium, auxin cytokinin interaction, and effect of wounding were studied on protocorm multiplication.

*p*H of the solid as well as liquid media was adjusted at 5.5–5.6. For solid cultures, the medium was gelled with 0.8% agar (Bacteriological grade, Sisco Laboratories, Bombay, India). All cultures were kept in continuous light of 900 lux at 25±2°C with 50–60% humidity.

Results

Effect of Different Carbon Sources on Protocorm Multiplication

All the carbon sources tested, except lactose did enhance the multiplication of protocorms. However, sucrose and maltose were superior to fructose, glucose and mannose in which relatively fewer cultures responded. Rate of multiplication as measured by the number of proliferated units per culture did not show significant differences among different treatments (table 1, figure 1) except in lactose.

Table 1 *Effect of different carbon sources on protocorm growth and multiplication*

Carbon source (2%)	% of cultures showing multiplication	Avg. rate of proliferation per culture
Sucrose	83·3	12·9
Maltose	91·7	10·5
Lactose	8·3	2
D-fructose	58·3	10
D-glucose	33·3	8
D-mannose	50	11

No. of replicates 12 Period of culture 60 days
Size of protocorm 1–1·2 mm

Table 2 *Effect of different levels of sucrose on protocorm multplication*

Sucrose	% of cultures showing response	Avg. rate of multiplication per culture
0	66·7	0
0·5	100	12·1
1·0	100	11·45
2·0	100	12·54
5·0	58·3	6·71
10·0	0	—

No. of replicates 12; Growth period 60 days;
Size of protocorm 1–1·2 mm

Effect of Different Levels of Sucrose

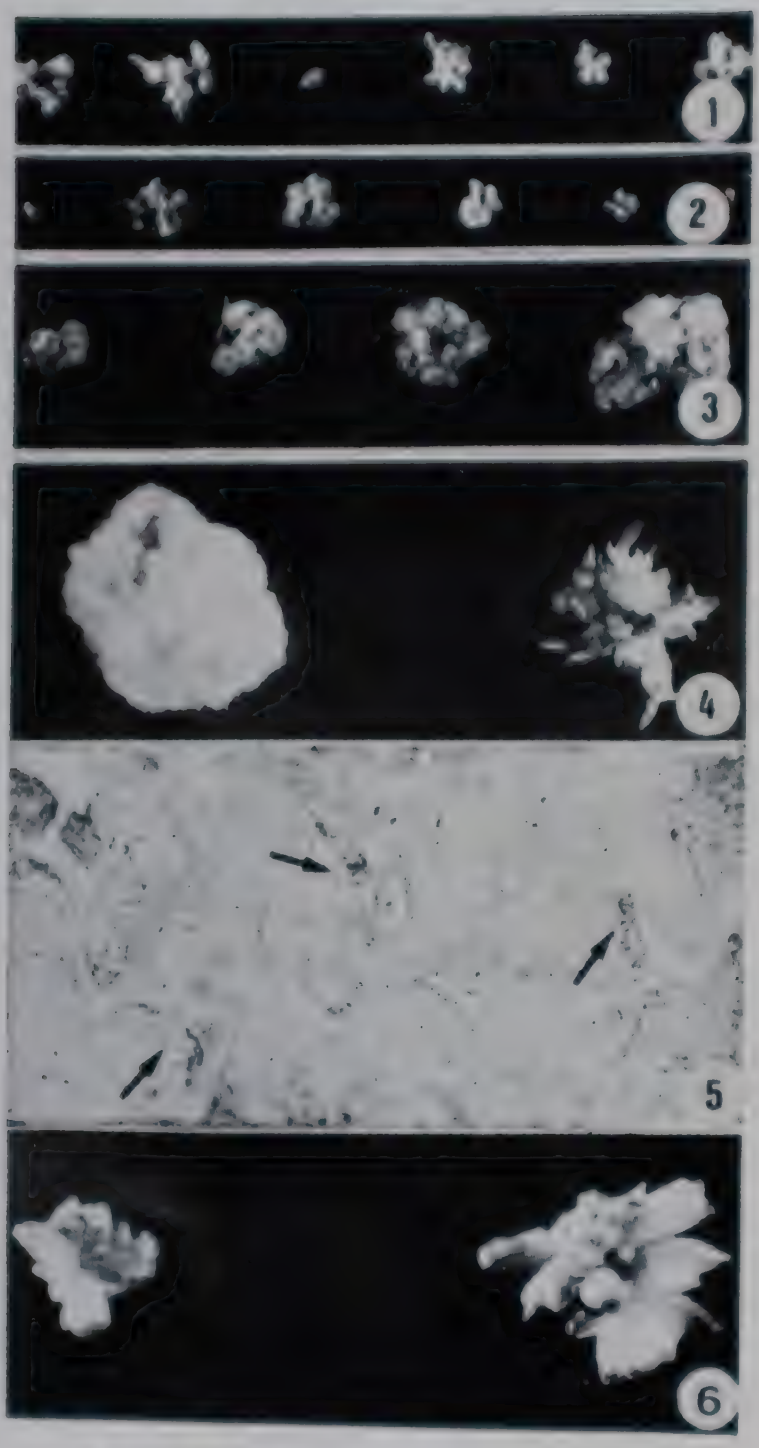
Results are summarised in table 2. The optimum levels of sucrose for maximum proliferation of protocorms was found to be 2%. Absence of sucrose stops growth of protocorms and 10% sucrose caused the tissue necrosis (figure 2).

Effect of Quantity of Medium

A direct correlation between the quantity of the medium supplied and rate of growth was evident in protocorm culture. Protocorms of uniform size were placed on modified basal medium in culture containers with different quantities of medium. The multiplication unit in flask culture with 72ml medium was thrice than that in small culture tube with 9 ml medium (figure 3).

Effect of Agitated Liquid Vs. Solid Medium

Protocorms 1 mm in size were cultured in shake flasks with liquid medium and in solid medium. Protocorms in liquid medium proliferated faster and appeared bigger than that in solid medium. However, the multiplied mass in liquid culture looked more an uneven callus mass of pale green colour compared to well defined green protocorms, grown on solid medium (figure 4).



Figures 1-6 **1**, Effect of different carbon sources on protocorm multiplication L-R, Sucrose, maltose, lactose, D-fructose, D-glucose and D-mannose (all at 2% concentration); **2**, Effect of different levels of sucrose on protocorm multiplication L-R, 0, 0.5, 1.0, 2.0, 5.0 and 10.0%; **3**, Effect of different quantities of medium on protocorm growth and multiplication L-R 9, 18, 36 and 72 ml.; **4**, Liquid vs solid culture on protocorm multiplication—note the callus like proliferation of protocorms in liquid culture (Left); **5**, Microtome sections of proliferation mass from agitated liquid medium—note the deeply stained cells forming like meristemoids at several parts of the parenchymatous tissues; **6**, Intact (L) and halved (R) protocorm on BM + IAA (1 ppm) + 2, i-P (1 ppm)

On prolonged culture, the tissues in liquid medium showed browning tendency. Histological sections of the proliferated unit in liquid cultures showed presence of deeply stained groups of cells (meristemoids) at several places of the callus-like mass (figure 5). Proper differentiation into protocorms was never seen.

Effect of Segmentation of the Protocorm on the Rate of Multiplication

Longitudinally divided halves of protocorms were individually cultured on modified medium with IAA (1 mg/l) + 2, *i*-P (1mg/l) to study the morphogenetic response of wounded protocorms. The average number of multiple units in this method was found to be 14.4 as compared to 7.2 obtained with culture of whole protocorms. Thus division of protocorms enhanced the rate of protocorm multiplication (figure 6).

Effect of Auxins on Protocorm

The response of protocorms to various auxin levels (0.5–10mg/l) is summarised in figure 7. All the auxins except 2,4-D favoured protocorm multiplication in the concentration range of 0.5–4 mg/l. IBA and BTOA enhanced the rate of multiplication up to 2 mg/l while decline was observed at 4 mg/l. In case of IAA and NAA the rate of proliferation showed a steady increase up to 8 and 6 mg/l respectively beyond which the proliferation rate decreased.

Effect of Cytokinins

Unlike the auxins, cytokinins (0.2–4.0 mg/l) in the medium did not enhance the rate of multiplication of protocorms or individual seedling growth.

Interaction of Auxin and Cytokinin on Protocorm Growth

The study on the effect of various auxins alone in the medium had revealed that IAA and NAA are the most favourable auxins

Table 3 *Effect of auxin-cytokinin interaction on protocorm multiplication*

Auxins (mg/l)	Cytokinin 2,i-P (mg/l)	1	2	4	8
IAA	1	+++	+++	+++	++
	2	+++	+++	+++	+++
	4	+++	+++	+++	+++
	8	+++	+++	+++	+++
	10	+++	+++	+++	+++
NAA	1	+++	+++	+++	+++
	2	+++	+++	+++	+++
	4	++	++	++	++
	8	++	++	++	++
	10	++	++	++	++

++ moderate; +++ high
Period of culture 65 days
No. of replicates 12

for protocorm multiplication. Hence combinations of IAA as well as NAA with 2,i-P or BA were tested. All the four combinations (i.e. NAA + BA, NAA + 2, i-P, IAA + BA, IAA + 2, i-P) induced protocorm multiplication at a wide range of concentrations 1–10 mg/l (tables 3, 4). 2,i-P at 10 mg/l was inhibitory irrespective of the auxin concentration and the protocorm showed a tendency to brown. BA in combination with various levels of IAA and NAA promoted protocorm multiplication even at 10 mg/l. BA and IAA or NAA combinations promoted callussing tendency along with differentiation while 2, i-P and auxin combinations always stimulated distinct protocorm formation.

Effect of CM

Two parallel experiments were conducted to study the effect of CM at various concentrations (5, 10, 15, 20 and 40%) on basal medium

Table 4 *Effect of auxin cytokinin interaction on multiplication of protocorms*

Auxins (mg/l)	Cytokinin (BA mg/l)	1	2	4	8	10
IAA	1	+++	+++	+++	+++	+++ CT
	2	+++	++	+++	+++	+++ CT
	4	+++	++	+++	+++	+++
	8	+++	++	+++	+++	+++
	10	+++	++	+++	+++	+++
NAA	1	+++	+++ CT	+++ CT	+++ CT	+++ CT
	2	+++	+++ CT	+++ CT	+++	+++ CT
	4	+++	+++ CT	+++ CT	+++	+++ CT
	8	+++	+++	+++	+++	+++ CT
	10	+++	+++	+++	+++	+++ CT

+ Less proliferation; ++ moderate proliferation; +++ high proliferation of protocorms;
CT callusssing tendency; Period of culture 65 days; No. of replicates 12

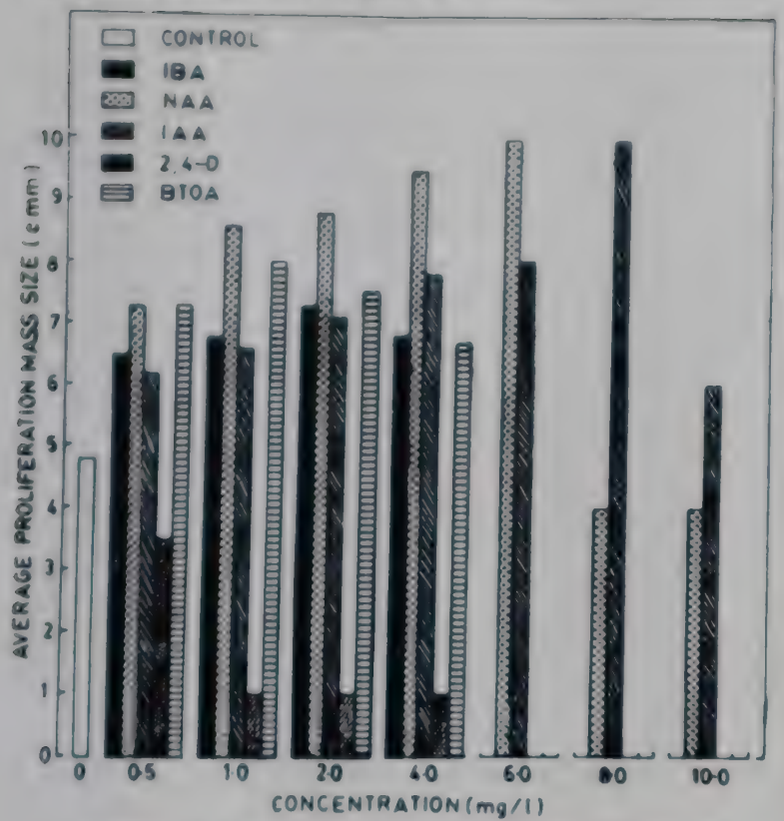


Figure 7 Histogram showing effect of various auxins on protocorm multiplication

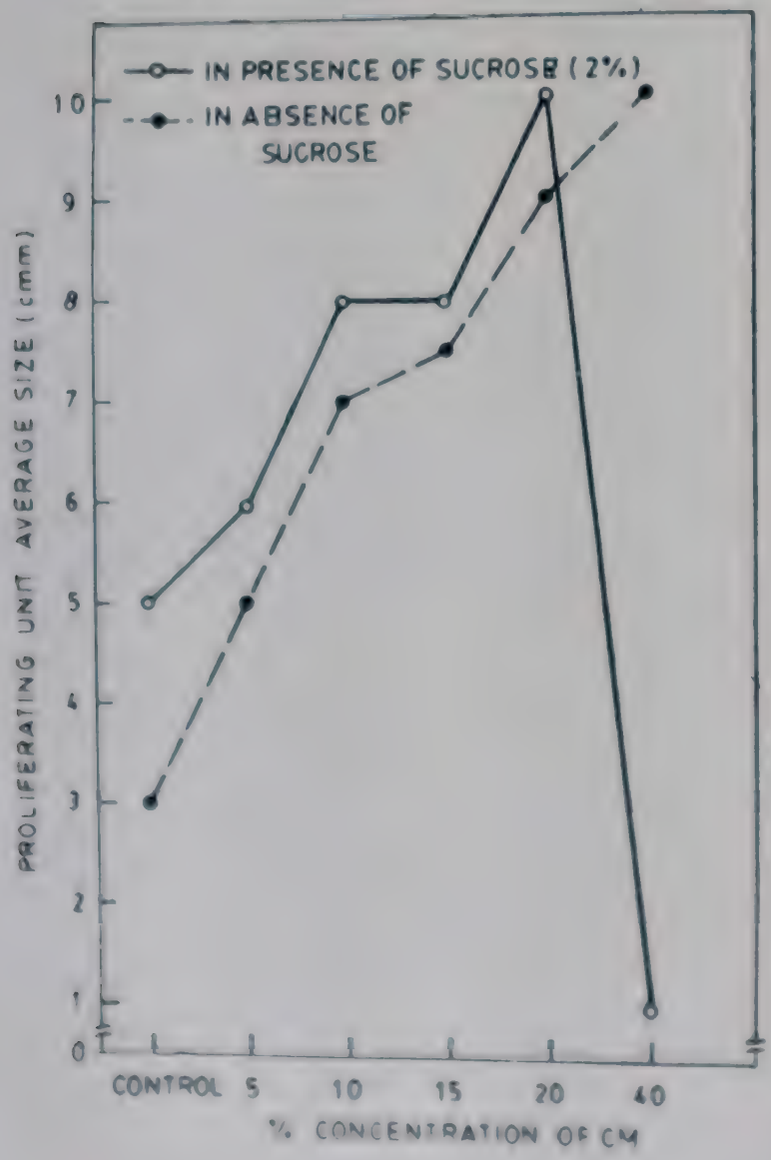


Figure 8 Graphic representation on the effect of CM in the presence and absence of sucrose protocorm multiplication

Figures 9-10 9, Response of protocorm explants of different sizes (5-7, 4.0, 2.0, 0.5 mm) to various media combinations BM, BM + IAA (1 ppm), BM + 2, i-P (1 ppm), BM + IAA + 2, i-p (1 ppm) BM + IAA + BA (1ppm); 10, Transplantable seedling growing on Nitsch basal medium

with sucrose 2% and the other on basal medium devoid of sucrose or any other carbon source.

The growth of protocorms showed a remarkable difference in their response to various levels of CM depending on the presence or absence of sucrose in the medium (figure 8). In sucrose containing medium 5–20% CM gave steady increase in rate of multiplication while in sucrose less medium with 5–15% of CM, the protocorm multiplication was slower. The protocorms were pale green, less defined and showed a tendency for callus formation. At 20% CM the sucrose-less medium showed well formed green protocorms like that in sucrose+ basal medium. When concentrations of CM was raised to 40% a very conspicuous difference in growth was observed with the presence or absence of sucrose. Explants became totally necrotic in sucrose+medium+ CM 40% while in sucrose-medium+CM 40%, enhanced protocorm proliferation was noticed.

Effect of GA

GA did not influence protocorm growth either alone or in the presence of auxins (IAA and/or NAA).

Explant Size and Culture Media

Studies involving the effect of auxins, natural extracts and cytokinins have shown that in almost all favourable media combinations protocorms undergo profuse proliferation. The next set of experiments were carried out to answer the following questions:

Under what growth conditions can isolated protocorms develop into seedling, multiply further units or undergo dedifferentiation? Is size of the protocorm at the time of culture critical for this type of growth?

In order to understand these parameters, an experiment was conducted using initial protocorm units of 4 different sizes: 0.5, 2.0, 4.0 and 5–7 mm length respectively. Protocorm explants of sizes 0.5 and 2.0 mm gave multiplication of protocorm units irrespective of the media used (figure 9). Four mm size explants generally underwent proliferation to form many protocorms in basal medium supplemented with IAA 1mg/l. With auxin and cytokinin combinations IAA and 2, i-P as well as IAA+BA, all at 1 mg/l, seedling growth was noticed along with proliferation of shoot base. When initial explant was 5–7 mm, development of individual plantlets was observed on the basal medium. At this stage (i.e. when explant was 5–7 mm) the addition of an auxin or cytokinin alone in the basal medium caused browning of differentiated leaves. A combination of auxin and cytokinin in the medium favoured proliferation and differentiation of protocorm units from the base of the developing plant. Therefore, for individual plant development, differentiated protocorms 5–7 mm could be cultured on basal medium alone.

Among the various basal media tested, Nitsch basal medium was the best for development of differentiated protocorms into complete plants (figure 10). Modified basal medium enchanced the seedling growth but with proliferation which could be checked by using half strength major elements. It was also observed that differentiated units if allowed to overcrowd for a longer period of time (4–5 months) without subculture, suffered from stunted growth. The optimum transfer period ranged from 70–80 days for proper growth of the plants.

Callus Studies on Protocorm Explants

Attempts to induce callus on protocorm explants with various concentrations and combinations of auxin and cytokinins did not give satisfactory results. Very often, dedifferentiation accompanied by protocorm differentiation was obtained. Callussing tendency was prominent at low level of

sucrose (0.5%) and could be maintained without differentiation by subculturing at 30 days interval to fresh medium.

When the medium was supplemented with IAA (2–8 mg/l) and 2, i-P or BA or Kn (at 2 mgl) the callus regenerated plantlets. The number of cultures showing differentiation varied in different treatments (table 5).

Discussion

In *Cymbidium* protocorms, sucrose is better than maltose, glucose and fructose (Fonnesbech 1972). In *Holttumara* Luke Tuck Yip—a vandaceous orchid, Teo and Wong (1978) recorded that sucrose, glucose and fructose are detrimental to the protocorm proliferation but not mannitol. In the present study 2% sucrose was ideal for multiplication of protocorms and in the absence of sucrose protocorms failed to grow.

Quantity of medium affected the rate of protocorm proliferation. The multiplying unit in flask culture with 72 ml of the medim was three times larger than in small culture tubes with 9 ml medium. Ichihashi (1978) has reported that the population density and volume of the medium were the deciding factors in *Bletilla* orchid seed culture. The influence of the size of the culture vessel on *in vitro* proliferation of grape was reported by Monette (1983), the highest number of shoots were observed in 473 ml jars followed by flasks of 250 ml and 125 ml.

In liquid medium, protocorms proliferated but did not produce well defined shoots. Kusumoto (1980b) reported that proliferation of protocorms could be stimulated in liquid media but that the formation of shoots and roots were retarded. Injury enhanced the multiplication rate in *Vanda* protocorms. Kusumoto (1978) reported that sections of protocorms were stimulated to undergo differentiation in *Cymbidium*. A large number of auxins favoured *Vanda* hybrid protocorm multiplication in a concentration range of 0.5–6.0 mg/l. 2, 4-D was an exception to this. IAA and NAA were effective even at 8 and 6mg/l. 2, 4-D induced abnormal growth and disturbed chlorophyll synthesis in *Cymbidium* (Fonnesbech 1972). This is contrary to the general finding in many monocotyledonous tissues where 2, 4-D is effective (Schenk & Hildebrandt 1972). In *Cymbidium* and *Cattleya*, a wide range of auxin cytokinin combinations was found favourable for protocorm proliferation (Kusumoto 1980b).

The enhancing effect of CM on protocorm multiplication had been studied by various orchidiologists (Vacin & Went 1949, Kusumoto 1969, Kusumoto & Furukawa 1977). Detailed studies with different concentrations of CM in the presence and absence of sucrose showed remarkable differences in their response. These differences could be attributed to the sugar content in the CM. When the basal medium contained 2% sugar and 40% CM, the total sugar content was too high and adversely affected the osmoregulation resulting in necrosis. When the medim was not supplemented with sucrose, the sugar content in 40% CM was appropriate as a carbon source and osmoregulator for growth of protocorms.

Table 5 *Regeneration in callus derived from protocorm*

Cytokinin	Cultures showing differentiation (%) and rate of differentiation, perculture		
	IAA mg/l 2mg/l	IAA 4 mg/l	IAA 8 mg/l
0	66·67%++	100++	50++
BA 2 mg/l	100++	100++	100++
2, i–P 2 mg/l	50++	33·33++	45·83++
Kn 2 mg/l	100+++	50++	83·33+

\+ low; ++ moderate; +++ high
No. of cultures 24; Period of culture 60 days

Application of GA did not enhance growth of *Vanda* protocorms. In tissue culture along with many reports on the positive action of GA (Kusumoto 1978, Smith 1968) the lack of response is also well documented in literature (Singh et al. 1974, Vieitez & Vieitez 1980).

The initial size of the explant as well as media were found to control the pattern of growth in *Vanda* whether the protocorm would develop into individual plant, or undergo protocorm multiplication or produce callus. In *Cymbidium* and *Cattleya* Mitra (1980) and Kusumoto (1980a, b) observed appreciable differences between culture conditions preferred for both protocorm proliferation and formation of shoot bud or root primordia in the protocorm.

Factors controlling growth of protocorms *in vitro* discussed here would be useful in selecting the media and stage of explants for rapid propagation of orchids.

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Intra-population Selective Mating as a Means of Improving Seed Yield in *Brassica campestris* var. Brown *Sarson*

G R DAS[1] and V ARUNACHALAM[2]

Division of Genetics, Indian Agricultural Research Institute, New Delhi 110012

(Received 27 November 1984)

Six populations of brown sarson (*Brassica campestris* L.) which were complex multiple crosses in their second generation were selected on the basis of genetic, geographic and general combining ability divergence of their parents. In each population, early and late plants were selected and raised to their next generation. In the early and late progeny families, several plants were full-sibbed to produce early and late sib-populations, EE and LL. In addition, phenotypically superior (High) female plants were pollinated with mixed pollen from phenotypically superior (High) or inferior (Low) plants to produce 4 categories of intra-population crosses—Early high × Late high, Early high × Late low; Late high × Early high, Late high × Early low. EE and LL sib-populations registered significant yield advance compared to their original counterparts. But the yield advances recorded by intra-population selective mating were much higher and consistent. The extent of yield improvement was proportional to the initial genetic variability in the populations. The results suggest that disruption of mating between intermediate types even within a genetically broad-based population will be a potent method to breed for enhanced productivity.

Key Words: *Brassica campestris* L., **Population improvement, Selective mating, Full-sib, Multiple crosses, Genetic advance**

Introduction

Brassica campestris is an important oleiferous crop consisting of three distinct cross-compatible varieties—brown *sarson*, yellow *sarson* and toria. Brown *sarson* contains self-incompatible, self-compatible and intermediate compatible genotypes. Yellow *sarson* is distinguished by its yellow seeds, bi- or multilocular siliquae and high clarity of oil. Early maturing and dwarf genotypes occur more often in toria. Cross-pollination mainly by insects and varying levels of incompatibility permit a variety of breeding procedures for

Present address: [1]Senior Breeder, Rice Research Station, PO Karimganj, Distt. Cachar, Assam
[2]National Fellow, Indian Agricultural Research Institute, Regional Station, Rajendranagar, Hyderabad 500030

enhancing the productivity of brown *sarson*. Nevertheless breeding efforts made so far have laid emphasis mainly on pedigree breeding method for evolving pure lines from single crosses. Earlier work in our unit has demonstrated the possibility of producing productive populations using multiple crosses (Arunachalam & Katiyar 1978, Bandyopadhyay & Arunachalam 1980). This paper reports further yield improvement in such populations by selective hybridisation.

Material and Methods

Six populations which were multiple crosses in their second generation were selected for the study. They had single crosses, biparental progeny or 3-way crosses as female parents and were pollinated earlier with mixed pollen from two or three cultivars (table 1). The female parent of population 1 (P1) was a single cross between brown and yellow *sarson*; that of population 2 (P2) was a single cross between a brown *sarson* line derived from disruptive and another from stabilising selection. Populations 3 and 4 (P3 and P4) had biparental progeny from a toria × brown *sarson* and a yellow *sarson* × brown *sarson* cross as their respective female parents. Populations 5 and 6 (P5 and P6) involved a female parent that was a 3-way cross of the type, (productive variant × original type) within a brown *sarson* cultivar, GBS2 crossed to another brown *sarson* cultivar, K1.

The multiple crosses producing the populations P1 to P6 had male parents which were mixed pollen from two or three cultivars of brown *sarson*, of which 71-6809 was from Canada, Bele, Torpe were from Sweden and others from India. Further, one parent of the multiple crosses had high and the other low overall general combining ability in their F_1 generation. Thus the parents of the crosses constituting the populations had geographic, genetic and general combining ability diversity. The populations P1, P2 had relatively low and P4, P5, P6 high variability.

In each of the population, eight early and eight late plants were selected and raised to their next generation. In the progeny families, several plants were full-sibbed to produce early and late sib-populations, to be denoted as EE and LL hereafter.

In addition, in the early and late progeny families, phenotypically superior plants were

Table 1 *Pedigree of the six populations*

Code	Pedigree	Parental description	Parental gca at multiple cross F_1 level
P1	(KL 17 × IB 3) × (Torpe + 71—6809)	SC × DP	H × L
P2	(DS—KL 17 × SS—PBST2) × (DS17D + PK + 184—63)	SC × TP	H × L
P3	(TL1842 × GBS2 (BIP F2)— 7—3) × (Bele + PK)	BP × DP	H × L
P4	(IB6 × DS17D (BIP F2)—2—1) × (Bele + PK + DS17D)	BP × TP	L × H
P5	(GBS2—B1—1—E3— PV × OR) × K1) × (Torpe + 71 — 6809)	TC × DP	H × L
P6	(GBS2—1—B1—1—E3—PV × OR) × K1) × (DS17D + PK + 184—63)	TC × TP	H × L

SC, Single cross; *BP*, Biparental progeny; *TC*, Three-way cross; *DP*, Pollen mixture of two cultivars; *TP*, Pollen mixture of three cultivars

identified as females. They were pollinated with mixed pollen from several phenotypically superior (high) or inferior (low) plants to produce four categories of intra-population crosses:

EL 1 = Early high × Late high
EL 0 = Early high × Late low
LE 1 = Late high × Early high
LE 0 = Late high × Early low

In the plants used as females, some branches were used for crosses and from some others parental seeds were collected at harvest.

The intra-population crosses, full-sibs and parents were grown in the next generation on ridges of 3 m length with a plant to plant distance of 10 cm. The number of ridges occupied by each treatment like EL1, EL0, full-sib etc. varied according to the available seeds. In each treatment, seed yields on two random samples of variable sizes from 30 to 100 plants were recorded. For purposes of analysis, the yields of samples were adjusted to a uniform size of 100 plants.

The crop received basal fertilizers of 40 kg N, 40 kg P_2O_5 and 40 kg K_2O/ha 40 kg. N/ha was top-dressed about 45 days after sowing. Irrigation and regular plant protection measures were given as usual.

The improvements in yields of intra-population crosses over their female parents and also the better of the early or late parental bulks, and of full-sib populations over the better of the early or late general bulk were calculated. The variation in yield advances (%) of the duplicate samples per plot were statistically analysed.

Results

The differences in yield advance over female parent by intra-population selective mating were significant among the four crosses and the six populations. The within population variances and the population × cross interaction were also significant (table 2). This means that the four types of crosses registered variable amounts of response in the populations (table 3). For instance, late high × early low cross caused 93% improvement in Population 1 while early high × late low crosses failed to bring about improvement in Population 2. Late high × early low and late high × early high crosses produced high yields in most of the populations. Late high females and high pollen were generally responsible for increasing yield. The % of crosses recording significant yield advance confirmed these results (table 3).

The yield advances in EE and LL sib-populations were significant (table 4). But the differences between EE and LL over the populations were not significant. The EE sib-population recorded an yield advance of 117.3% in P4 followed by P2 and P5 (36%). The yield advances recorded in the corresponding LL sib-populations were, however, consistent (53 to 89%). The most responsive population to full-sibbing was P4 and the least responsive was P1 (table 5).

Table 2 *ANOVA of yield improvement over female parent by intra-population selective mating*

Source	d.f.	m.s.
Treatments	23	5005.3*
Populations	5	9163.9*
Crosses	3	7760.9*
Early vs. Late female	1	17323.9*
EL1 vs. ELO	1	5946.5*
LE1 vs. LEO	1	12.3
Populations × Crosses	15	3068.0*
Within treatments	137	3317.8*
P1	27	7562.5*
P2	28	3590.8*
P3	25	1640.9*
P4	17	2299.4*
P5	22	2517.3*
P6	18	795.3*
Error	161	459.0

*Significant at 5% level

Table 3 *Number of intra-population crosses (n) made in the 6 populations, and those (%) recording significant yield advance (p) and magnitude of improvement (%) over female parent (q)*

		Mating system				Female		Pollen		Weighted mean
		EL1	EL0	LE1	LE0	Early	Late	High	Low	
P1	n	10	5	11	5	15	16	21	10	
	p	40.0	20.0	45.5	60.0	33.3	50.0	42.9	40.0	42.0
	q	38.7*	21.5*	38.9*	93.1*	32.9*	55.9*	38.8*	57.3*	44.8*
P2	n	10	5	12	5	15	17	22	10	
	p	30.0	20.0	50.0	60.0	26.7	52.9	40.9	40.0	40.6
	q	12.9*	−4.7	39.6*	39.1*	7.0	39.5*	27.5*	17.2*	24.3*
P3	n	11	5	8	5	16	13	19	10	
	p	36.4	20.0	62.5	40.0	31.3	53.8	47.4	30.0	41.4
	q	19.5*	1.4	44.2*	23.9*	13.8*	39.4*	29.9*	12.6*	23.9*
P4	n	4	5	7	5	9	12	11	10	
	p	0	40.0	0	20.0	22.2	8.3	0	30.0	14.3
	q	5.9	24.1*	9.7	20.5*	16.0*	14.2*	8.3	22.3*	15.0*
P5	n	8	4	9	5	12	14	17	9	
	p	62.5	25.0	44.4	40.0	50.0	42.9	52.9	33.3	46.1
	q	38.8*	12.9	33.8*	19.0*	30.1*	28.5*	36.2*	16.3*	29.3*
P6	n	6	5	6	5	11	11	12	10	
	p	16.7	0	50.0	0	9.1	27.3	33.3	0	18.2
	q	5.7	3.3	12.3*	0.5	4.6	6.9	9.0*	1.9	5.8
Over-all	n	49	29	53	30	78	83	102	59	
	p	34.7	20.7	43.4	36.7	29.5	41.0	39.2	28.8	35.4
	q	22.4*	9.6*	32.1*	32.7*	17.7*	32.3*	27.5*	21.4*	25.2*

*Significant at 5% level

The yield improvement by early×late (= EL1 + EL0) or by late×early (= LE1 + LE0) intra-population mating showed significant differences among the six populations. early×late and late×early matings produced significant differences in yield advance over the populations (table 6). Population 4 gave again the best response to intra-population mating followed by P2, P3 and P5. Population 1 recorded a negative response (table 7).

The most responsive population P4 was obtained from a cross whose female parent was a biparental progeny from a yellow× brown *sarson* cross and male was a pollen mixture of three varieties, one from Sweden and two from India. The least responsive population P1 was from a cross whose female parent was a single cross of the type brown× yellow *sarson* and male was a pollen mixture of two exotic cultivars (table 1). Thus the initial genetic variability present in the population P4 was far greater than that in P1. Populations P2 and P5 originated from multiple crosses of the type, single cross× pollen from 3 cultivars and 3-way cross× pollen from 2 cultivars. Their genetic base, though broad, was only next to population P4. Thus the level of response to intra-population selective mating or full-sibbing was proportional to the composition and diversity of the initial genetic base.

Table 4 *ANOVA of yield advances due to sibbing*

Source	d.f.	m.s.
Treatments	11	7220.0*
EE	5	9836.3*
LL	5	5969.4*
EE vs. LL	1	391.6
Error	12	336.8

*Significant at 5% level

Table 5 *Yield advance in populations due to sibbing*

	EE	LL	mean	
P1	—27.9	—63.0*	—45.4*	*e*†
P2	36.0*	53.3*	44.6*	*bc*
P3	15.4	14.4	14.9	*d*
P4	117.3*	88.8*	103.0*	*a*
P5	35.9*	75.9*	55.9*	*b*
P6	17.4	36.2*	26.8*	*cd*
Mean	32.3*	34.3*	33.3*	

†Values carrying identical letters do not differ significantly
*Significant at 5% level

Table 6 *ANOVA of yield advances due to selective hybridisation*

Source	d.f.	m.s.
Treatments	11	6082.9*
EL	5	6924.2*
LE	5	6442.6*
EL vs. LE	1	77.7*
Error	12	11.0

*Significant at 5% level

Table 7 *Yield advance due to intra-population selective hybridisation*

	EL	LE	mean
P1	—29.5*	—19.2*	—24.3*
P2	66.9*	76.0*	71.5*
P3	49.8*	57.2*	53.5*
P4†	146.6*	141.0*	143.8*
P5	38.5*	51.6*	45.0*
P6	14.4*	1.8*	8.1*
Mean	47.8*	51.4*	49.6*

†Advance due to EL not significantly different from that to LE; *Significant at 5% level

Discussion

Most of the successful varieties in cereals initiate from a broad genetic base. Such a base was created by the multiple crosses that involved high levels of genetic, geographic and combining ability diversity. Selection for flowering time within such diverse populations established extremes even for yield, as was shown earlier too (Ram et al. 1969, Murty et al. 1972). The broad initial genetic base sustained sufficient variability within the moities selected for early and late flowering in each population. Full sib-mating broke linkage disequilibrium and released new recombination (Kojima & Kelleher 1963, Miller & Rawlings 1967, Sprague 1971). This could be a plausible explanation for yield advance under sib-mating.

Intra-population hybridisation in populations descending from multiple crosses one of whose parents had high and the other low overall general combining ability generated a high frequency of productive recombinants. They compared well with the recorded productive potential of dent×flint, dent×flour and flint×flour crosses in maize (Richey 1922), high×low crosses (Hull 1952 in maize; Langham 1961 in sesamum) and winter×spring crosses in wheat (Mckenzie & Grant 1974). The selection of early or late mother

plants and high or low pollen provided another dimension of disruptive and negative assortative mating within populations to increase productive recombinations further (see also Thoday 1972). The disruptive mating between early and late extremes gave, as expected, higher yield improvement than recorded by full-sib mating within early or late extremes. The extent of improvement was proportional to the initial genetic variability as was evident by a comparison of yield advances in populations P4, P5, P3 and P1.

The results suggest that disruption of mating between intermediate types even within a genetically broad-based population would shuffle desirable genes from the extremes to produce a new potential source of variability. Repeated cycles of such intra-population selective mating should result therefore in a uniform and highly productive population within a reasonably short time. This technique is hence worthy of practice in toning up the productivity of *Brassica campestris* as demonstrated by this study.

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Cation-induced Changes in Fluorescence Yield, and Emission of *Synechococcus* spheroplasts: Effect of Monovalent, Divalent and Trivalent Cations

R A WAVARE and PRASANNA MOHANTY
School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067

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$AlCl_3$ either present in the algal growth medium or exogenously supplied to *Synechococcus cedrorum* spheroplasts induced a blue shift in the Chl *a* peak emission while no change in the phycocyanin emission was induced. Like Al^{3+} ions, La^{3+} also caused similar change in the room temperature emission spectrum. In contrast, the addition of Na^+, K^+ or Mg^{2+} to spheroplasts did not alter the spectral characteristics.

All the ions, however, brought about a small decrease in the intensity of chlorophyll *a* emission, and the magnitude of lowering depended on the charge of the ion. The order of decrease in the fluorescence intensity was as follows: $Al^{3+} > La^{3+} > Mg^{2+} > K^+$. The addition of Al or La did not bring about changes in the extent of energy transfer from phycobilins to chlorophyll *a*. Thus the changes induced in the Chl *a* emission by Al^{3+} or La^{3+} appears to be associated with alteration in the lipid protein interactions of cyanobacterial membranes.

Key Words: **Absorption, Emission, Excitation, Quenching, Blue shift, cation effect, *Synechococcus***

Introduction

Measurements in Chl *a* fluorescence yield and emission of chloroplasts and algal cells provide information of absorption, distribution and utilization of energy in photosynthesis. Chl *a* emission characteristics have been used as a probe for studying the redistribution of excitation energy between the two photosystems (Papageorgiou 1975, Lavoral et al. 1977, Williams 1977), heterogeneity of photosynthetic apparatus, the redox changes and pool size of electron transport carriers as well as variety of other enegy linked functions of chloroplasts (Papageorgiou 1975). Recent studies on cation induced Chl *a* fluorescence yield changes have revealed the mode and extent of energy transfer between two photosystems and the possible ways in which cations regulate optimal rate of photosynthesis (Homann 1969, Murata 1971, Gross & Hess 1973, Mohanty et al. 1973, Mills & Barber 1978). Most of the studies on cation induced changes of structure and function have been made

Abbreviations: Chl *a*: Chlorophyll *a*; PC : Phycocyanin; PS I : Photosystem I; PS II : Photosystem II. Tes N-tris (hydroxymethyl) methyl-2-eminoethane sulphonic acid

with intact or broken chloroplasts. Only a few studies on cation induced changes of photosynthesis have been carried out with cyanobacterial cells (Ono & Murata 1978, Piccioni & Mauzerall 1978, Brand 1979). Mohanty et al.(1974) have shown that addition of chloride or acetate of K^+ or Mg^{2+} quenched the room temperature chlorophyll fluorescence in *Chlorella*. These results are in contrast to the results obtained in broken chloroplasts where Mg^{2+} increased the fluorescence yield in diuron (DCMU) poisoned chloroplasts (Homann 1969, Murata 1969, and Mohanty et al. 1973). We have, therefore, attempted to characterize the change in absorption and emission spectra of cyanobacterial spheroplasts as affected by the addition of K^+, Na^+, Mg^{2+}, Al^{3+} and La^{3+}. Also attempts have been made to characterize the fluorescence spectra of spheroplasts prepared from the cells grown in the presence of $AlCl_3$.

Materials and Methods

Cells of cyanobacterium: *Synechococcus cedrorum* were grown in continuous light of 1000 lux in the presence and the absence of 0.3 mM AlCl 3 at 27°C as described earlier (Wavare & Mohanty 1982). The cells were harvested by centrifugation at late log phase after 6 days and washed once in the fresh culture medium. The cells were suspended in 20 mM Tricine-KOH (*p*H 7.5) containing 400 mM sucrose, 10 mM KCl and 10 mM EDTA and washed and suspended in the same buffer and incubated at 37°C for 3 hrs with 1 mg/ml lysozyme according to Peter and Sherman (1978) for spheroplast preparation.

Measurement of Room Temperature Chl a Fluorescence

The room temperature Chl *a* fluorescence emission spectrum and relative fluorescence yield of spheroplasts were measured as follows. The sample was excited with a high pressure mercury lamp (HQE-40, West Germany). The light from the lamp was passed through a filter (CS 4-72). The emission of Chl *a* fluorescence was measured at right angle to the excitation beam with an EMI photomultiplier with S20 response operated at 1200 V. The fluorescence light before reaching the photomultiplier was passed through a cut off filter (CS 2-64) and a monochrometer (MDR II, Russian, 600 lines/mm). The output of the photomultiplier was fed to an electrometer amplifier (ECIL, India, Model EA B 12) and then to the X-Y recorder. For measurement of Chl *a* fluorescence, spheroplasts were incubated in 3 ml reaction mixture containing 15 mM TES-KOH (*p*H 7) buffer, 10mM KCl, 400 mM sucrose and 4.5 μg Chl *a*/ml according to Biswal et al. (1979). The sample was excited at 436 nm for Chl *a* excitation and 560 nm for phycocyanin (PC) excitation.

Measurement of Absorption Spectra of Spheroplasts

The room temperature absorption spectra of spheroplasts of *Synechococcus* were measured by a Hitachi double wavelength/ double beam spectrophotometer (Model 557, Japan). The *Synechococcus* cells grown for 6 days were used. Salts were added 5 min before measurement.

Results and Discussion

Changes in Chl a Fluorescence Emission of Spheroplasts by Addition of Cations

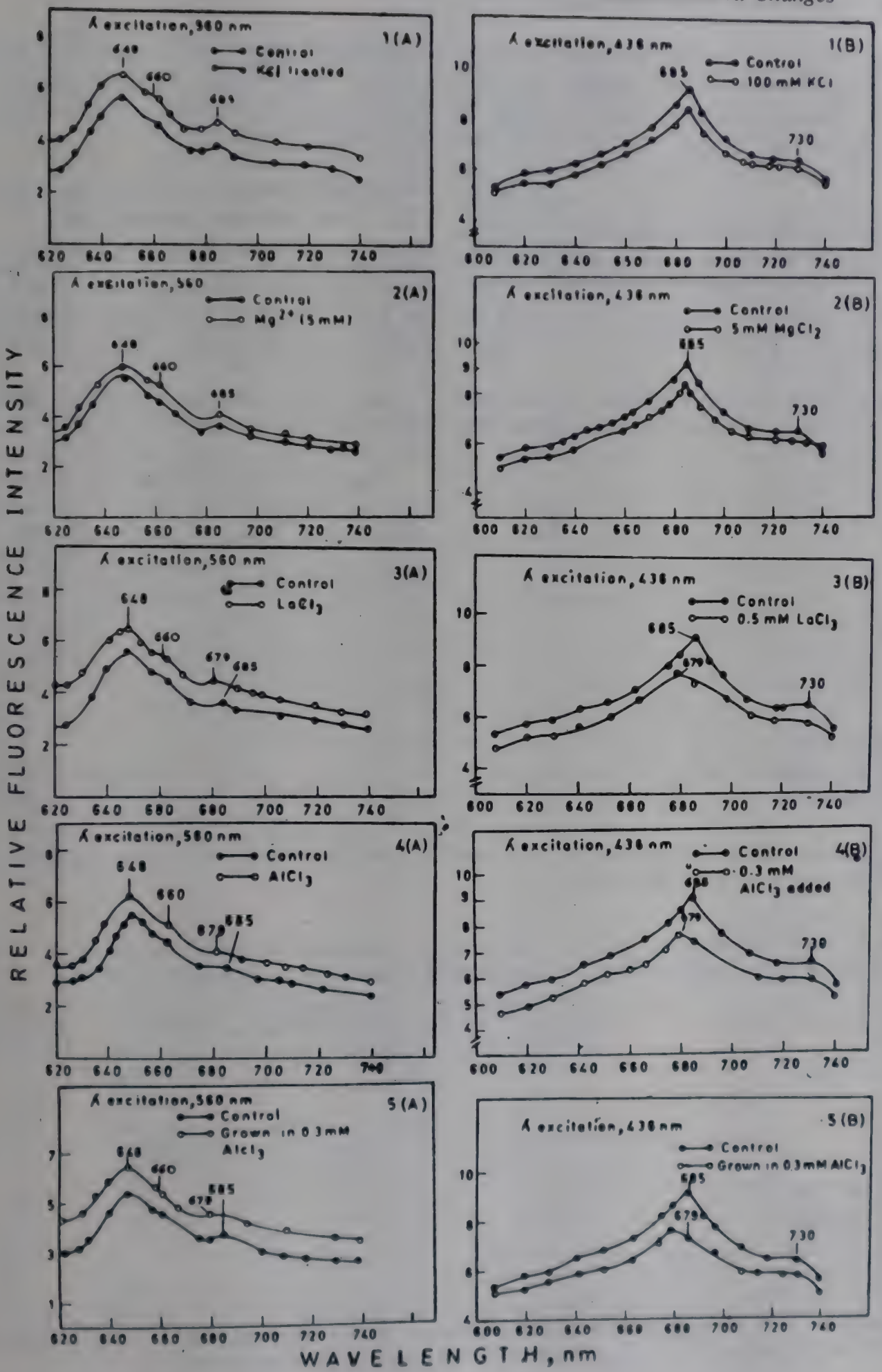
We have measured the room temperature fluorescence emission spectra of spheroplasts in the presence and absence of exogenous cations, excited at two different wavelengths of excitations (i) at 560 nm which is mainly absorbed by phycobillisomes, and (ii) at 436nm which is absorbed by weakly fluorescent forms of Chl *a* (Duysens 1963).

With *Synechococcus* spheroplasts, the room temperature fluorescence spectrum, upon excitation with green light (560nm)

showed a peak at 648 nm (emission due to c-phycocyanin) and shoulder at 660 nm (emission due to a allophycocyanin) and a relatively small band at 685 nm (F_{685}) due to emission from Chl *a*. The spheroplasts show intense PC fluorescence and small band due to Chl *a* fluorescence which suggests a decrease in the extent of energy transfer from PC to Chl *a* II. Figure 1A shows that the addition of K^+ (or Na^+) to the spheroplasts only slightly changed the intensity of emission. Thus addition of these cations did not alter the emission characteristic of spheroplast either when excited at 560 nm (figure 1A) or at 436 nm (figure 1B) except that the presence of the salt reduced the emission intensity at 685 band (figure 1B). Similarly the room temperature (298K) emission spectra of spheroplasts measured in the presence and absence of 5mM $MgCl_2$ revealed similar characteristic emission profiles (figures 2A, B). It appears that cations like Na^+or K^+, Mg^{2+} lowered the fluorescence intensity at 685 (F_{685} band) (figure 2B). Figure 3 shows the effect of trivalent cation La^{3+} on fluorescence spectrum excited at 560 nm and 436 nm. Addition of this cation caused a shift in Chl *a* II emission band from 685 to 679 (figures 3A,B) and this shift in the emission peak position appeared more prominent when excited at 436 nm (figure 3B), than when 560 nm excitation was used (figure 3A). No change in peak position of phycocyanin emission is observed due to the addition of La^{3+}. Figure 4 (A,B) shows with spheroplasts a similar shift in the peak emission position from 685 nm to 679 nm. Along with the Al^{3+} induced shift in the peak position, we also observed a lowering of fluorescence intensity at 685 (F_{685}) nm. Figure 5 (A,B) shows similar changes in the Chl *a* peak emission when cells were grown with $AlCl_3$. Figure 6 shows the extent of lowering of relative fluorescence intensity by addition of various concentration of KCl, $MgCl_2$, $LaCl_3$ or $AlCl_3$. The extent of lowering of fluorescence intensity initially increased with increase in the concentration of salts and then it remained constant. The extent of quenching was maximum with Al^{3+}, and the order of effectiveness in inducing the quenching of Chl *a* fluorescence was $Al^{3+} > La^{3+} > Mg^{2+} > Na^+$ or K^+. Table 1 indicates the ratios of relative fluorescence intensities emitted at different wave length of emission upon 560 nm excitation. The data show that the addition of La^{3+}, Al^{3+} or Ca^{2+} did not alter the ratio of F648/F685 or F660/685 suggesting no further change in energy transfer from PC to Chl *a* II, while K^+ or Na^+ caused a small decrease in the ratio. There is, however, change in the magnitude of the ratio of F660/F679 by Al^{3+} or La^{3+} as would be expected due to shift in the peak position by these ions (figures 3,4). If fluorescence emission at 648 (F648) were normalised (excitation, 560 nm), one observes a quenching of F 685 band by addition of ions; K^+ or Na^+ induced higher quenching than Al^{3+} or La^{3+} and

←

Figures 1–5: 1. Effect of addition of 100 mM monovalent cations (KCl or NaCl) on room temperature Chl *a* fluorescence emission spectra of spheroplasts; λ excitation for (A) – 560 nm and (B) – 436 nm. The 3 ml reaction mixture used for all assays contained 400 mM sucrose, 10 mM KCl, and 4·5 μg Chl/ml; 2. Effect of 5 mM $MgCl_2$ on room temperature Chl *a* fluorescence emission spectra of spheroplats; λ excitation used for (A) – 560 nm and (B) – 436 nm; 3. Changesin room temperature Chl *a* fluorescence emission spectra of spheroplasts of *Synechococcus* by the addition of 0·5 mM $LaCl_3$; λ excitation used for (A) – 560 nm and (B) – 436 nm; 4. Changes in room temperature Chl *a* fluorescence emission spectra of spheroplast by incubation with 0·3 mM $AlCl_3$; λ excitation for (A) – 560 nm and (B) – 436 nm; 5. Changes in room temperature Chl *a* fluorescence emission spectra of spheroplasts of *Synechococcus* grown in presence and absence of 0·3 mM $AlCl_3$; λ excitation used for (A) – 560 nm and (B) – 436 nm


λ excitation, 560 nm
1(A)
Control
KCl treated
648
660
685
λ excitation, 438 nm
1(B)
Control
100 mM KCl
685
730
λ excitation, 560
2(A)
Control
Mg2+ (5 mM)
λ excitation, 438 nm
2(B)
Control
5 mM MgCl2
λ excitation, 560 nm
3(A)
Control
LaCl3
679
λ excitation, 438 nm
3(B)
Control
0.5 mM LaCl3
λ excitation, 580 nm
4(A)
Control
AlCl3
λ excitation, 438 nm
4(B)
Control
0.3 mM AlCl3 added
λ excitation, 580 nm
5(A)
Control
Grown in 0.3 mM AlCl3
λ excitation, 438 nm
5(B)
Control
Grown in 0.3 mM AlCl3
RELATIVE FLUORESCENCE INTENSITY
WAVELENGTH, nm

Mg^{2+} and Ca^{2+} which induced the least quenching. These results suggest that K^+ or Na^+ caused a slightly larger decrease in the extent of energy transfer from PC to Chl *a* II than Al^{3+}, La^{3+} of Ca^2 or Mg^{2+} in *Synechococcus* spheroplasts. Similarly, table 2 catalogues the data on the changes of the relative ratio of F685/F730 emission bands; when samples were excited in blue light ($\lambda = 436$ nm). The extent of lowering of F_{685} band is 30% for Al^{3+}, La^{3+} and 25% for Mg^{2+} or Ca^{2+} and 20% for K^+ or Na^+. The change in the magnitude of F_{685}/F_{715} thus is found to be maximal for Al^{3+} and least for K^+.

Fluorescence Emission of Spheroplasts from Cells Grown with and without $AlCl_3$

Since addition of Al^{3+} ions induced maximal quenching and alteration in spheroplast, we have measured the Chl *a* fluorescence emission characteristic of spheroplasts

Table 1 *Effect of addition cations on the ratio of fluorescence intensities emitted at* 685(F_{685}), 679 (F_{679}), 660 (F_{660}) and 648 (F_{648}) *nm of spheroplasts of* Synechococcus cedrorum

	Ratio of relative intensity				
	$\frac{F_{648}}{F_{685}}$	$\frac{F_{660}}{F_{685}}$	$\frac{F_{648}}{F_{679}}$	$\frac{F_{660}}{F_{679}}$	Extent of quenching of F_{685}
None	2·3	1·7	2·8	2	—
KCl (100 mM)	1·8	1·36	—	—	16
NaCl (100 mM)	1·95	1·47	—	—	16
$MgCl_2$ (5mM)	2·1	1·75	—	—	7
$CaCl_2$ (5 mM)	2·1	1·75	—	—	7
$AlCl_3$ (0·3 mM)	2·3	1·7	—	1·6	8–10
$LaCl_3$ (0·5 mM)	2·2	1·66	—	1·58	9–10

λ Excitation = 560 nm; For comparison of the extent of quenching of F_{685} by cations the fluorescence intensities at 660 nm were normalised

Table 2 *Effect of addition of cations on the ratio of fluorescence intensities emitted at* 685 (F_{685}), 679 (F_{679}), 715 (F_{715}) and 730 (F_{730}) *nm of spheroplasts obtained from* Synechococcus cedrorum

Salts (in mM)	Ratio of fluorescence intensities			Quenching of F_{685}
	$\frac{F_{685}}{F_{715}}$	$\frac{F_{685}}{F_{730}}$	$\frac{F_{679}}{F_{730}}$	%
None	3	3·66	3·2	—
KCl (100 mM)	2·8	3·18	—	20
$MgCl_2$ (5 mM)	2·7	3·1	—	25
$AlCl_3$ (0·3 mM)	2·6	2·9	3·5	30
$LaCl_3$ (0·5 mM)	2·7	3	3·4	27

λ Excitation = 436 nm, and emission slits were 2 nm; spectra were recorded at room temperature. Fluorescence intensity at 685 nm (F_{685}) without the salt addition was taken as 100%

isolated from cells grown in the presence and absence of $AlCl_3$ (0.3 mM). The results obtained with 6 day old cells is shown in figure 5. The emission spectrum strangely enough showed a shift in the Chl *a* II peak emission by 5–6 nm as it is seen when Al ions are added to spheroplasts (figure 4). The extent of lowering fluorescence intensity remained, almost about the same extent or slightly less when $AlCl_3$ is exogenously added. Thus presence of Al ions during growth medium caused nearly similar change in emission spectra (figure 5B). These results suggest that Al ions are taken up by cells and these ions interact with protein pigments of the photosynthetic membrane.

We have previously shown that the addition of Al to the growth medium changed the absorption characteristic of spheroplasts (Wavare & Mohanty 1982). We have measured the effect of addition of cations on absorption spectra of spheroplasts, and compared the ratio of absorption at 620 nm (due to PC) to that at 679 (due to Chl *a*). It is found that the peak ratios A_{620}/A_{679} for control and in presence of K^+ or Mg^{2+} remained same whereas the peak ratio A_{620}/A_{679} for Al^{3+} or La^{3+} was 0.97 of control. This shows that addition of cations did not change the peak ratios significantly suggesting that there is no direct interaction of phycocyanin with these metal ions.

In summary, addition of $AlCl_3$ or $LaCl_3$ in the growth medium or exogenously added to of *Synechococcus cedrorum* spheroplasts caused a lowering of Chl *a* fluorescence intensity and changed the position of peak emission. A blue shift of 5–6 nm in Chl *a* fluorescence emission spectrum as due to the presence of Al^{3+} ions suggests an alteration in the membrane pigment protein interactions. (figure 4A, B, 5A, B). Although spectral shifts were seen with trivalent Al^{3+}, monovalent and divalent cations (K^+ and Mg^{2+}) did not bring about any change in the peak emission.

These salt-induced changes may be, in part due to a change in the mode of excitation energy transfer between the pigments. The ratios of F_{685} (Chl *a* II)/F_{730} (Chl *a* I) at room temperature were decreased by the addition of cation (table 2). This possibly indicates that cations increase energy transfer from

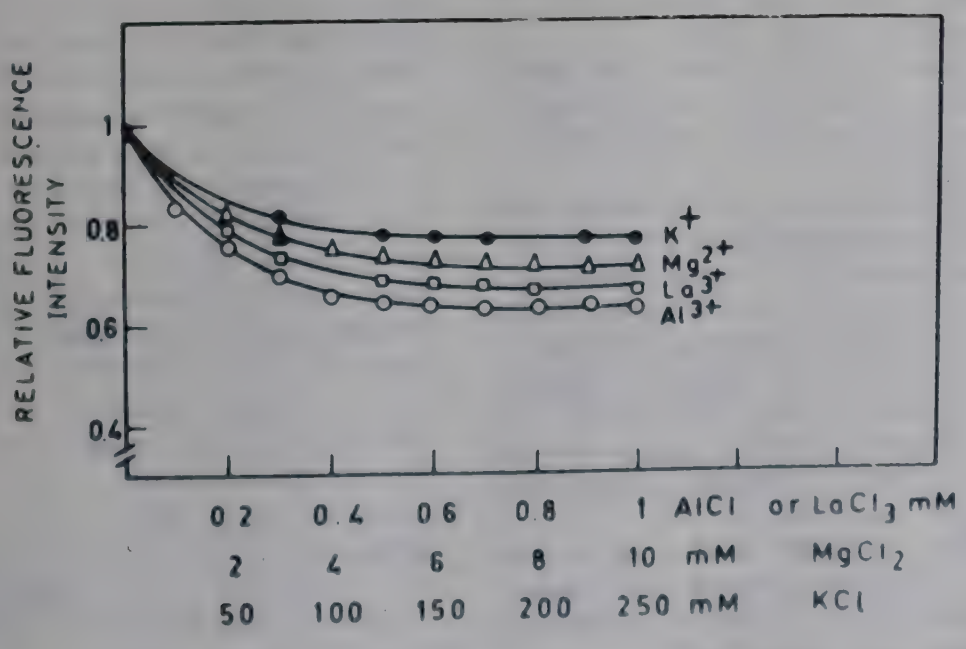


Figure 6 Effect of varying concentration of cations (K^+, Mg^{2+} La^{3+}, Al^{3+}) on room temperature Chl *a* fluorescence intensity of spheroplasts

system II to system I as suggested by Mohanty et al. (1974) who observed the quenching of Chl *a* fluorescence yield by KCl, $MgCl_2$, K-acetate and Na-acetate in *Chorella* cells but in contrast to salt-induced increase fluorescence yield observed with broken chloroplasts (Homann 1969, Murata 1969, Mohanty et al. 1973). The cation-induced changes in absorption in Chl *a* and fluorescence peak by Al^{3+} and La^{3+} appear to be irreversible occurring through specific binding of the cation to spheroplasts membrane protein pigment because of high electrostatic attraction exerted by the negatively charged membrance surface (Mills & Barber 1978). However, addition of cations like Al^{3+} and La^{3+} did not seem to alter the ratios F_{648}/F_{685} or F_{660}/F_{685} suggesting that the presence of these metal ions possibly do not affect the phycobilisomes. The analysis of various membrance pigment proteins from cells grown with and without Al^{3+} and La^{3+} ions will be an useful tool for probing the structure and function of cynobacterial membranes.

Acknowledgement

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A Model to predict Wheat Emergence from Soil Temperature and Soil Moisture

A S SETHI[1], R P MOR[2] and G C AGGARWAL[3]
Punjab Agricultural University, Ludhiana

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The seedling emergence of 2 wheat varieties at various temperatures and soil moisture levels was evaluated. Mean emergence (over moistures and temperatures) was related to time through a Mitscherlich equation. A second degree logistic growth model was developed for predicting wheat emergence at a given time from temperature and soil moisture. An exponential model which provides a temperature-moisture schedule for achieving a desired emergence in a certain time was also developed. Validation of the logistic model has been confirmed from a good agreement found between the observed and predicted values of emergence under naturally varying temperature conditions.

Key Words: Logistic growth model, Varietal emergence difference in wheat, Sensitivity to temperature and moisture

Introduction

Due to increase in cropping intensity, sowing of wheat is done under considerably varying environmental conditions from October to early January. Wheat emergence and consequently crop stand is primarily affected by temperature and moisture (Hillel 1972, Lindstrom et al. 1976). Models capable of predicting wheat emergence under given temperature and moisture regimes will help in achieving the desired crop stand by manipulation of the seed rate or soil moisture content as temperature can be estimated from meteorological data. Lindstrom et al. (1976) model is of limited utility as it predicts time for 50% emergence only, neither higher nor lower emergence. we have followed a new approach in the development of models capable of predicting (*i*) emergence of wheat at a given time under different environmental conditions and (*ii*) optimum soil-water content for achieving desired crop stand in a given time under the prevailing temperature regime.

Materials and Methods

Theory

For computational convenience, the equally

1 Sugarcane Research Station, Jalandhar;
2 Haryana Agricultural University, Hissar;
3 Department of Soils, Punjab Agricultural University, Ludhiana

spaced temperature T (in °C) and soil moisture content θ (in %) were transformed into x and y as

$$x = (T - 10)/3 \text{ and } y = (\theta - 9)/2$$

Emergence ratio (defined as the emergence at any time as a fraction of the maximum attainable emergence, averaged over all temperature and moisture levels at t days after sowing, $\bar{p}_t$, was related to time t (in days after sowing) through a Mitscherlich equation written as:

$$\bar{p}_t = a - b\ exp\ (-ct) \quad .. \quad (1)$$

where a, b and c are the unknown parameters of the equation. Following the approach of difference equations (Nair 1964) the equation was expressed linearly as:

$$D_t = a' + b' S_t \quad .. \quad (1.1)$$

where $D_t = (\bar{p}_{t+1} - \bar{p}_t)$, $S_t = (\bar{p}_t + \bar{p}_{t+1})$ and $a' = -2ab'$.

The coefficients a' and b' in (1.1) could be easily estimated, thus furnishing the estimate of a. The equation (1) could then be rewritten as :

$$Log\ (a - \bar{p}_t) = Log\ b - ct \quad .. \quad (1.2)$$

which was linear in Log $(a - \bar{p}_t)$ and t, from where the least squares estimates of b and c were evaluated. Equation (1) provides only an approximate estimate of the time (t_0) needed for achieving a desired emergence ratio $(\bar{p}_{t_0})$ as it ignores the influence of moisture and temperature on the emergence time.

Following Landsberg (1977) emergence ratio $(p_{x,y})$ at time t_0 for temperature x and moisture y was expressed as second degree logistic growth model in x and y, viz.

$$p_{x,y} = k/\{1 + f\ exp\ (-g_1 x - g_2 y + g_{11} x^2 + g_{22} y^2 + g_{12} xy)\} \quad .. \quad (2)$$

representing geometrically a sigmoidal surface with a fall. The parameter k, which denotes the maximum attainable value of the dependent variable, equals 1.0 in the present case, the highest value of emergence ratio. Then the growth model (2) could be expressed as :

$$Log\ (1/p_{x,y} - 1) = Log\ f - g_1 x - g_2 y + g_{11} x^2 + g_{22} y^2 + g_{12} xy \quad .. \quad (2.1)$$

which represents the usual second degree multiple regression equation relating Log $(1/p_{x,y} - 1)$ with x and y. From (2.1) the parametric quantities f, g_1, g_2, g_{11}, g_{22} and g_{12} of the model (2) could be easily estimated. Equating $p_{x,y}$ in (2) to $\bar{p}_{t_0}$ and simplifying, a solution of y in terms of x was found to be

$$y = \{(g_2 - g_{12} x) - (l x^2 + mx + n)^{1/2}\}/2g_{22} \quad .. \quad (2.2)$$

Where,

$$l = g_{12}^2 - 4 g_{11} g_{22},$$
$$m = 2 (g_2 g_{12} - 2 g_1 g_{22}),$$
$$n = g_2^2 + 4 g_{22} \dot{p}_{t_0}, \text{ and}$$
$$\dot{p}_{t_0} = Log\ \{(1/p_{t_0} - 1)/f\}$$

Applying elementary algebra, it can be seen that the range of x in (2.2) providing real solutions for y is given by

$$x = \{-m \pm (m^2 - 4\ ln)^{1/2}\}/21 \quad .. \quad (2.3)$$

The equation (2.2) was used for computing various pairs of real values of moisture (y) and temperature (x) which would result in a predetermined emergence ratio $(\bar{p}_{t_0})$ after a given time (t_0). For facilitating the estimation of the corresponding values of moisture and temperature for the desired emergence, the above obtained values were further related through an exponential function (Draper & Smith 1968), given by

$$y = u\ exp\ (-vx + wx^2) \quad .. \quad (3)$$

The unknown parameters u, v and w of the model (3) were estimated from its parabolic expression (3.1) between Log y and x, viz.

$$Log\ y = Log\ u - vx + wx^2 \quad .. \quad (3.1)$$

This model provides a temperature-moisture schedule for achieving a crop stand of desired density in a certain time.

Goodness of each model fitted to two wheat varieties was evaluated by computing a measure ϕ defined as 1.0 minus the ratio

of the residual sum of squares to the corrected total sum of squares (Ware et al. 1982).

Experimental

A laboratory study was conducted to study the effect of 6 soil moisture and 6 constant temperature levels on emergence time of 2 wheat varieties. Surface silty clay loam (sand 21.5%, silt 45.4% and clay 32.8%) was air dried, sieved, and wetted in bulk to water contents ranging from 11 to 21%, in 2% increments. Seeds of two wheat varieties (Shailaza — a two gene dwarf and local — a tall variety) were planted at a depth of 4 cm in soil adjusted to different water contents and packed into 1 litre plastic containers. The containers were covered with polyethylene sheet with two small holes to allow adequate air exchange and were placed in a temperature controlled room. Temperature was changed between different sets within the range of 13 to 28°C with 3 degree increments and was maintained within ± 0.5°C. The required temperature was achieved in the insulated room by using room heaters connected in series with an adjustable thermostat. Air was circulated with a table fan, placed near the room heater, for maintaining uniformity in room temperature. Each set was replicated three times. Seedling emergence was recorded daily up to 10 days after sowing.

In the second phase of the study, emergence time under naturally varying temperature conditions was observed for evaluating the predictability of this model in the field. In this study containers were removed to a well ventilated laboratory. Variation in temperature was achieved by sowing the seeds on four different dates — at an interval of 10 days. A thermograph was placed in the laboratory for recording temperature variations. Temperature varied from 22 to 27°C, 18 to 27°C, 19 to 24°C and 17 to 22°C during the 4 different sets of observations. Mean temperature of these 4 different sets of observations was 24.9, 22.0, 20.7 and 19.6°C. Soil moisture levels were same as those of first experiment.

Results and Discussion

Model 1

Estimates of the unknown parameters of this model for the two varieties are tabulated below:

Variety	a	b	c	ϕ value
Local	0·9485	2·1866	0·3489	0·998
Shailaza	0·8001	2·3732	0·3190	0·996

Average emergence for local wheat is consistently more than that for Shailaza (figure 1). Very high values of ϕ reveal a nice goodness of the fitted model. For local wheat, expected $\bar{p}_t$ was 0.758 at 7 DAS and 0.854 at 9 DAS. However, the expected $\bar{p}_t$ for Shailaza wheat was 0.546 at 7 DAS and 0.666 at 9 DAS.

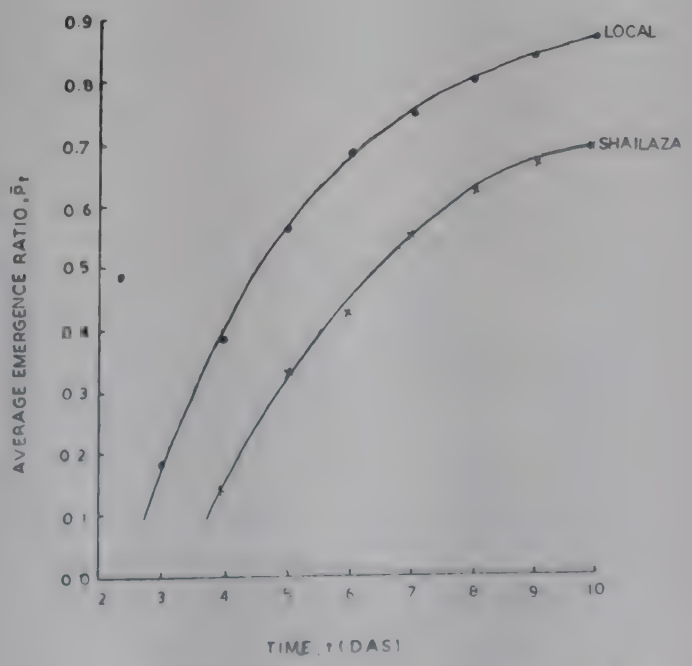

Figure 1 Average emergence of Local and Shailaza varieties of wheat in relation to time

Variety	f(x10⁶)	g_1	g_2	g_{11}	g_{22}	g_{12}	ϕ value
Local	2·2944	5·7179	4·3408	0·5704	0·3633	0·2904	0·923
Shailaza	0·4668	5·4177	2·6408	0·5884	0·1970	0·2019	0·878

Model 2

Fitting this model to both the varieties at $t = 9$ DAS yielded the above set of estimates of the parameters:

Fairly high values of ϕ, each computed at 36 data points, indicate goodness of fit of the model 2, which jointly relates emergence ratio to temperature and soil moisture after a known time.

A relative comparison of emergence ratio (observed and predicted) for both the varieties at 9 DAS conspicuously reveals that sigmoidal surface for the local variety is consistently higher than that for the Shailaza variety (figure 2), indicating thereby that the combined effect of temperature and moisture on local wheat is more pronounced than that on Shailaza. However, the sensitivity analysis based on computing values of partial derivatives of $p_{x,y}$ with respect to x and y revealed that except for 3 combinations of T and θ, viz. (13°C, 11%), (13°C, 13%) and (16°C, 11%), the values for Shailaza were higher than those for the local. This implies that barring a few low-levelled combinations of T and θ, Shailaza variety is more sensitive to changes in temperature and soil moisture than local wheat.

Model 3

Fitting this model for $t = 9$ DAS for both the varieties yielded the following set of estimates of the unknown parameters:

Variety	u	v	w	Expected emergence ratio ($\bar{p}_{t_0}$)	ϕ value
Local	19·5272	1·3409	0·1468	0·854	0·965
Shailaza	182·0056	2·5251	0·3001	0·666	0·915

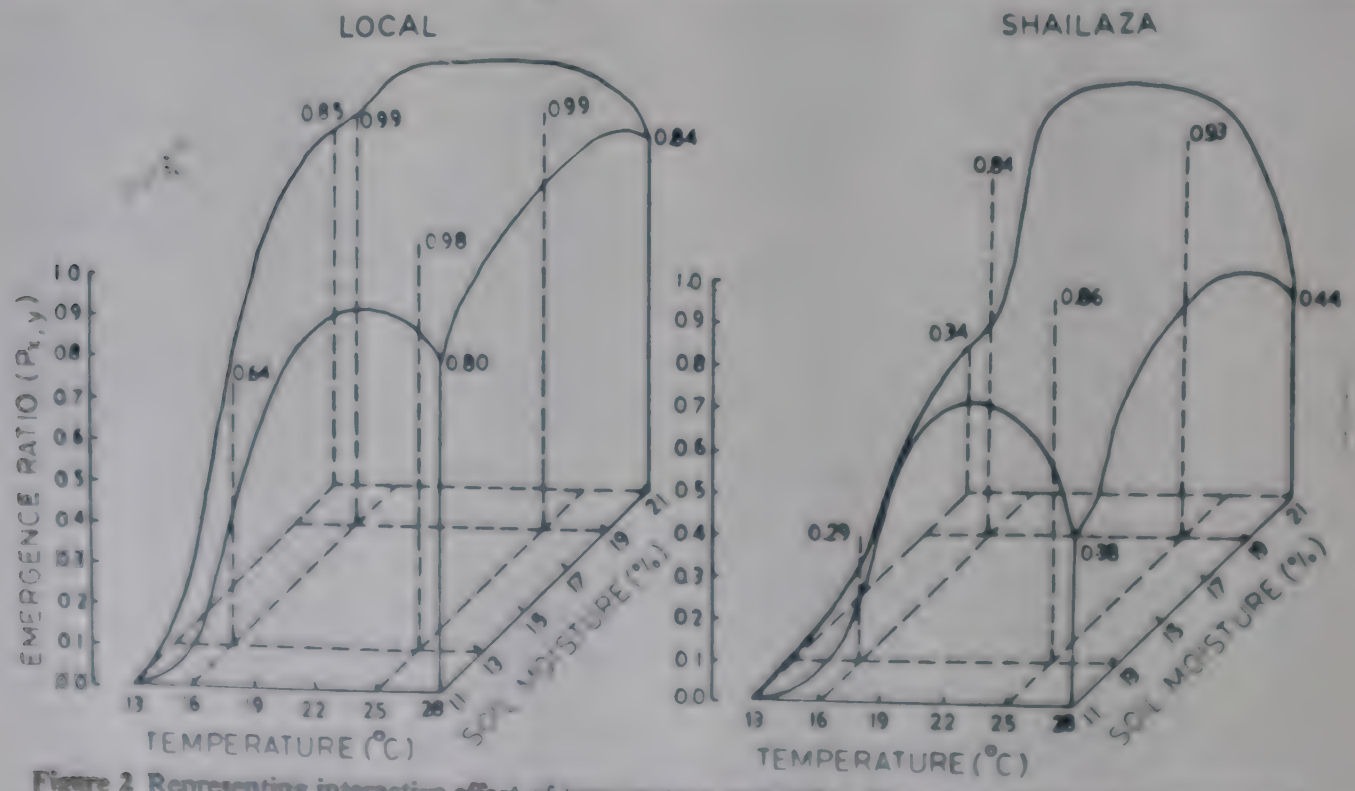

Figure 2 Representing interactive effect of temperature and soil moisture on emergence percentage of Local and Shailaza wheat at 9 DAS

Again, substantially high values of ϕ confirm goodness of fit of this model as well. The temperature-moisture schedule for both the varieties have been presented graphically (figure 3).

Finally, to assess applicability of the model 2 under varying temperature (field) conditions, estimated emergence was calculated for $t = 7$ and 9 DAS, using average of the variable temperature in the model. A fairly close proximity between observed and the corresponding predicted values (figure 4) indicates applicability of the model under varying temperature (field) conditions as well.

This model predicts that 91% emergence in local wheat can be achieved 7 days after sowing (data not included) when soil moisture content is 11% and temperature is 22°C. The same emergence can also be obtained in the same period by ensuring 17% moisture content when temperature is expected to be 16°C. Thus this model provides management practices for obtaining the desired crop stand under different situations. This model also predicts environmental conditions for ensuring early targetted emergence. For example 88% emergence of Shailaza wheat 9 days after sowing when soil moisture content is 21% and temperature is 25°C, could also be achieved in 7 days, according to this model, provided 15% soil moisture content and 25°C temperature is maintained. This model (Equation 2.3) also gives a critical temperature range within which the desired emergence can be obtained by maintaining certain level of soil moisture. This model predicts that 67% emergence of Shailaza wheat 9 days after sowing can be achieved only within the temperature range of 14.5 to 28.2°C. Outside this critical temperature range, the desired emergence cannot be achieved by manipulation of the soil moisture.

Development of crust after wheat sowing may adversely affect emergence and consequently utility of this model. Encrustation during wheat emergence period, however, is unlikely as soil drying is slow and probability of rains is low during this period. Another limitation of this model

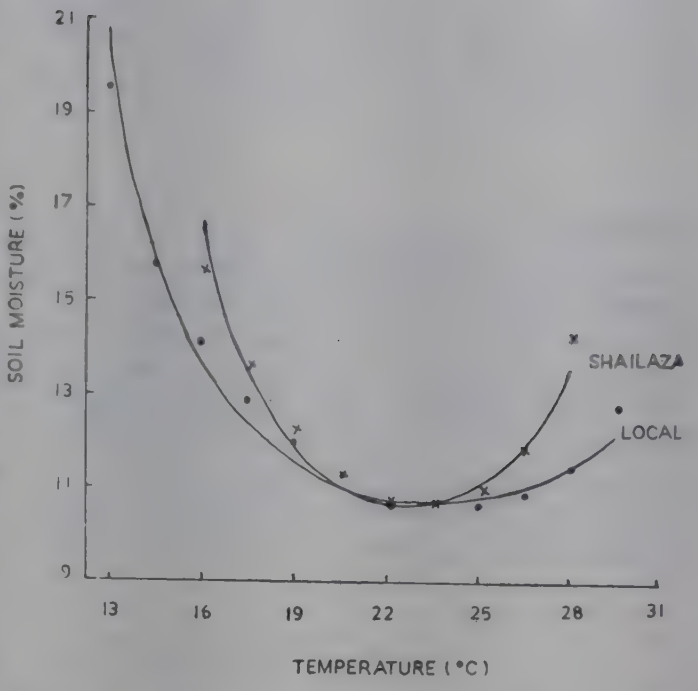

Figure 3 Temperature-moisture relationship for 85% emergence of Local and 66.6% emergence of Shailaza wheat at 9 DAS

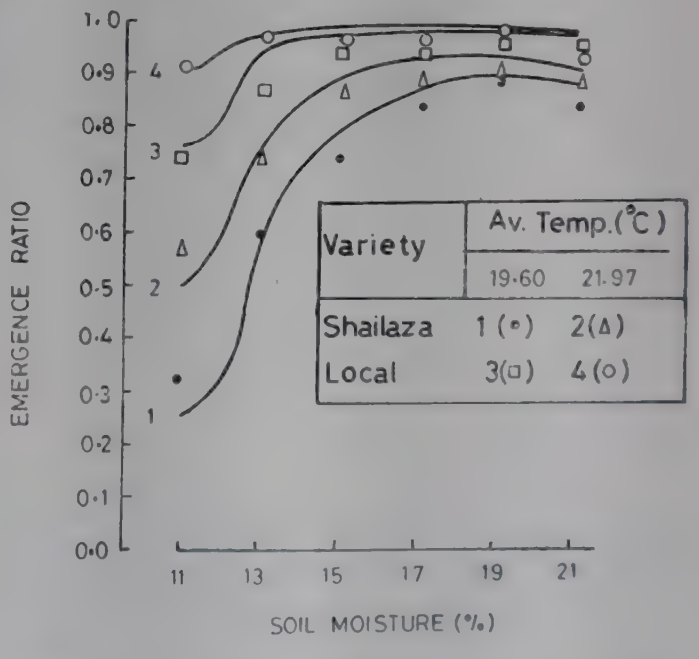

Figure 4 Observed and predicted emergence of Local and Shailaza varieties of wheat under variable temperature at 7 DAS

is that it does not apply equally well for the entire range of temperature and moisture tested, though the overall predictability of the model is fairly high. Predictability is relatively more at higher levels of moisture and temperature.

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Effect of Clipping on Growth and Total Non-structural Carbohydrates of *Diplachne fusca* in Saline and Alkali Soils

ANUBHA SINHA, S R GUPTA* and R S RANA**
Department of Biosciences, Maharshi Dayanand University, Rohtak 124 100

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Effect of intensive and multiple clipping on regrowth and total non-structural carbohydrate content of *Diplachne fusca* was studied under saline and alkaline soil conditions to explore the usefulness of the grass under combined conditions of heavy grazing and salt-stress. Experiments were conducted under controlled conditions in pots using alkali soils (*p*H 9.3 and 9.8), saline soils (electrical conductivity, EC_e 6 and 12 ds/m) and normal soil (EC_e 0.5 ds/m; *p*H 7.5). Plant vigour and yield declined significantly after two clippings. Clipping influenced the total non-structural carbohydrate (TNC) content in aboveground and belowground parts of the plants and regrowth of plants was related with TNC reserves of residual plant tissues after clipping. Growth performance of the grass was superior in alkali soils than in other soils.

Key Words: Alkali soil, Clipping, Herbage yield, Saline soil, Total non-structural carbohydrates

Introduction

In the arid and semi-arid regions of India, there are millions of hectares of land affected by saline and alkali soils, which support a sparse growth of vegetation. A major part of these marginal lands in rural areas are under heavy grazing pressure because these lands mainly fall under "community lands" and serve as common grazing facilities for livestock. Perennial grasses are important under such conditions because they have a potential to regenerate and regrow after being defoliated or clipped. Belowground reserves of these grasses are probably exploited for regrowth of shoots after clipping (Alcock 1964, Ehara et al. 1966). The main source of energy for regrowth and maintenance of plant growth is usually suggested to be in the form of total non-structural carbohydrates present in the plant tissues (Dwyer et al. 1963, Smith & Marten 1970, Trlica & Cook 1971, Bokhari & Singh 1974).

Keeping in view these considerations,

*Department of Botany, Kurukshetra University, Kurukshetra 132 119
**Central Soil Salinity Research Institute, Karnal 132 001

the present study was aimed at evaluating the growth performance of *Diplachne fusca* L P Beauv. in saline and alkali soils, under intensive and multiple clipping. Effect of clipping and soil type on total non-structural carbohydrates (TNC) of the plants was also studied. *Diplachne fusca* is a palatable, perennial grass which grows naturally in salt-affected soils and has been reported to possess a marked salt tolerance, particularly to alkalinity (Rana & Parkash 1980, Sinha et al. 1982).

Materials and Methods

The experiment was conducted under controlled conditions using pot culture. The study extended for a period of 12 weeks during August to October, which is the major growth period of the grass under natural conditions.

Bulk samples of normal soil (*p*H 7.5; electrical conductivity EC_e, 0.5 ds/m) and alkali soils (*p*H 9.3, *p*H 9.8) were obtained from natural grassland sites of Kurukshetra, and two levels of soil salinity (EC_e 6 and 12 ds/m) were prepared by mixing salts of NaCl, $CaCl_2$ and Na_2SO_4 in normal soil, following USDA Handbook No. 60 (1954).

Ten tillers of *Diplachne* were transplanted in each pot (15 cm high, 18 cm diameter) containing 4 kg of one of the five soil types. A total of 75 pots were grouped as 5 soil types × 3 clipping treatments × 5 replicates. Clipping treatments comprised of unclipped plants serving as control (C_0), and plants clipped intensively at 2 cm height once at 4-wk stage (C_1), and twice at 4-wk and 8-wk stages, (C_2). The pots were watered daily to 50% of total water holding capacity. Biomass and other growth parameters (height, tillers, leaf area) were measured at the time of each clipping and final harvest at 12-wk stage. All plant samples were dried at 80° C and weighed. Total non-structural carbohydrates (TNC), comprising the sugars readily available for growth were estimated on dry weight basis by acid hydrolysis followed by Shaeffer-Somogy Copper Iodometric titrations (Smith 1969).

All the data were subjected to t-test and two-way analysis of variance for analyzing the effect of clipping treatment in different soil types.

Results

Clipping reduced the vigour of plants in all the soils, the reduction being more pronounced for the plants subjected to two clippings. Although there was an overall decrease in vigour in terms of height, leaf area and biomass, but clipping resulted in increased tillering in all the soils.

Herbage yield (dry weight basis) showed a progressive decline at each successive cutting (table 1). Total cumulative yield also decreased due to multiple clipping. Results of t-test indicated a significant ($P < 0.01$) decline in herbage yield only under C_2 (twice-clipped) treatment as compared to C_1 (once clipped) and C_0 (unclipped). Two-way analysis of variance indicated that aboveground and belowground biomass of the plants varied significantly due to soil type [$F_{0.01}$ (df 4,8) = 130.5, 142.5] and clipping treatment [$F_{0.01}$ (df 2, 8) = 210.0, 246.2] but not due to the interaction of the two factors, soil type × clipping. This indicates that the effect of clipping treatment was similar in different soil types within 5% limits of probability. Cumulative herbage yield was found to be more in plants growing in alkali soils and normal soil than in saline soils (table 1).

Effect of clipping on TNC content of aboveground and belowground parts of the grass varied markedly (table 2). Cumulative TNC yield (g/pot) of aboveground parts and TNC reserves of belowground parts decreased with increased clipping and in all the soils the decrease was

Table 1 *Biomass of* Diplachne *under different clipping treatments in normal, saline and alkali soils*

Soil type	Clipping[a] treatment	Herbage yield (g/pot)				Belowground biomas (g/pot)
		4 wk	8 wk	12 wk	Cumulative (Mean±1 s.e.)	12 wk (Mean±1 s.e,)
Normal	C_0	—	—	29·6	29·6±0·90	17·6±0.72
(EC_e 0.5 ds/m *p*H 7.5)	C_1	8·4	—	16·2	24·6±1·08	15·8±0·86
	C_2	8·4	7·6	3·8	19·8±0·50*	12·1±0·40*
Saline I	C_0	—	—	21·6	21·6±1·00	18·2±0·85
(EC_e 6 ds/m)	C_1	6·0	—	14·0	20·0±0·98	17·1±0·78
	C_2	6·0	4·0	3·6	13·6±1·20*	14·5±0·90*
Saline II	C_0	—	—	22·6	22·6±0·72	16·2±0·54
(EC_e 12 ds/m)	C_1	5·6	—	13·8	19·4±0·81	15·4±0·60
	C_2	5·6	4·0	3·4	13·0±0·95*	12·5±0·85*
Alkali I	C_0	—	—	30·6	30·6±1·22	20·8±1·00
(*p*H 9.3)	C_1	13·0	—	15·0	28·0±0·96	19·9±0·92
	C_2	13·0	7·6	2·0	22·6±1·00*	18·1±1·11*
Alkali II	C_0	—	—	30·8	30·8±1·50	21·2±1·10
(*p*H 9.8)	C_1	12·0	—	17·8	29·8±1·35	18·5±1·00
	C_2	12·0	6·4	2·4	21·0±1·11*	18·0±0·92*

[a]C_0, unclipped; C_1, once clipped; C_2, twice clipped
*The value is significantly ($P>0.01$) lower than that of unclipped C_0 treatment

statistically significant ($P < 0.01$) between C_2 treatment and other clipping treatments, as shown by t-test. Two-way ANOVA indicated significant differences in TNC content due to soil type [$F_{0\cdot01}$ (df 4,8)=59.9] as well as clipping [$F_{0\cdot01}$ (df 2, 8)=108.0] but not due to their interaction ($P > 0.05$). Clipping treatments also influenced the allocation of TNC in aboveground and belowground parts of the grass. At final harvest (12-wk), it was found that belowground TNC reserves were more than aboveground TNC content, under C_2 treatment, whereas the reverse was the case under C_0 treatment in all the soils (table 2).

Discussion

Multiple clipping of aboveground parts significantly reduced the herbage yield of the grass, because of frequent removal of potential photosynthetic surface area. As a result of intensive clipping at two centimeter height, there occurred a decrease in cumulative yield also. It is perhaps because after each clipping the plants had to expend appreciable energy as respiration losses and also due to very little residual assimilatory surface left after each clipping. Similar reductions in cumulative yield after intensive defoliation have been reported by Holscher (1945), Dwyer et al. (1963) and Buwai and Trlica (1977) for some other grasses. The trends of variations in TNC contents of aboveground and belowground parts under different treatments clearly indicate some relationship between TNC content and regrowth of the plants. A gradual decline in belowground biomass and belowground TNC reserves of the grass with increasing clipping suggests

Table 2 *Total non-structural carbohydrate (TNC) content in aboveground and belowground parts of* Diplachne *under different clipping treatments in normal, saline and alkali soils*

Soil type	Clipping[a] treatment	TNC (g/pot) Aboveground parts				Belowground parts
		4 wk	8 wk	12 wk	Cumulative (Mean ± 1 s.e.)	12 wk (Mean ± 1 s.e.)
Normal	C_0	—	—	5·73	5·73 ± 0·20	1·49 ± 0·10
(EC_e 0.5 ds/m *p*H 7.5)	C_1	1·67	—	3·00	4·67 ± 0·21	1·01 ± 0·20
	C_2	1·67	1·25	0·50	3·42 ± 0·25*	0·82 ± 0·35*
Saline I	C_0	—	—	2·04	2·04 ± 0·02	1·46 ± 0·16
(EC_e 6 ds/m)	C_1	0·91	—	1·82	2·73 ± 0·19	0·82 ± 0·09
	C_2	0·91	0·55	0·43	1·89 ± 0·03	0·62 ± 0·25*
Saline II	C_0	—	—	3·03	3·03 ± 0.11	1·17 ± 0·12
(EC_e 12 ds/m)	C_1	0·73	—	1·63	2·36 ± 0·15	1·05 ± 0·05
	C_2	0·73	0·42	0·35	1·50 ± 0·17*	0·56 ± 0·05*
Alkali I	C_0	—	—	6·08	6·08 ± 0·25	1·79 ± 0·03
(*p*H 9.3)	C_1	2·36	—	2·52	4·88 ± 0·22*	1·43 ± 0·02
	C_2	2·36	1·10	0·28	3·74 ± 0·19*	1·23 ± 0·10*
Alkali II	C_0	—	—	6·22	6·22 ± 0·27	2·17 ± 0·05
(*p*H 9.8)	C_1	2·61	—	3·40	6·01 ± 0·30	1·39 ± 0·08
	C_2	2·61	1·13	0·37	4·11 ± 0·31*	1·18 ± 0·04*

[a] C_0, unclipped; C_1, Once clipped; C_2, Twice clipped

*The value is significantly ($P < 0.01$) lower than that of unclipped C_0 treatment

an upward translocation of carbohydrate reserves to the regenerating aboveground parts after each clipping.

Thus, it follows that *Diplachne* can tolerate at least one intensive clipping in normal, alkali, as well as saline soils without any significant reduction in cumulative herbage yield. Carbohydrate reserves in belowground parts of the grass appear to play a significant role in regeneration and regrowth of the shoots after clipping. Belowground TNC reserves are reduced markedly after second clipping, due to which regeneration and regrowth are retarded. The plants growing in alkali soils have relatively larger TNC content in belowground parts which is perhaps responsible for better growth and regrowth of the shoot in these soils. Superior growth performance of *Diplachne* under alkaline soil conditions and its good regrowth potential after clipping suggest that it may serve as a useful forage grass particularly in alkali soils.

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Bamboo in the Rural Life of Hill Tracts of the Western Ghats

S NARENDRA PRASAD and MADHAV GADGIL
Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012

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Bamboos are an important component of the forests of the Western Ghats, and must have played a significant role in the life of people since time immemorial. The earliest uses for people in the hunting-gathering stage must have included the construction of hunting implements and shelter, and use of shoots and seeds as food. When agriculture was taken up, bamboo began to play an important role in the construction of various agricultural implements, grain storage bins and the more elaborate houses of the farming communities. The use of bamboo by the farming communities is illustrated by a detailed survey of an Uttara Kannada village. The pastorals depend on bamboo as fodder especially in the summer when the grass dries up. In the simpler tribal and pastoral communities bamboo is worked by all members. However, in the more complex agricultural society some communities devote themselves entirely to bamboo working. In years of seeding, seed collection may provide subsistence to many rural communities. The modern use of bamboo in industry dates from the last half century. Prior to that bamboo was considered a weed by foresters; now that it is a valuable commodity, its availability for the local population has steeply declined due to over exploitation. This poses considerable hardships to the rural population.

Key Words: **Bamboo, Western Ghats, Basket-weavers, Paper mills**

Introduction

The hill chain of the Western Ghats runs parallel to the West Coast between the latitudes 8°N to 20°N. It experiences rainfall regimes ranging from 7500 mm to as little as 800 mm a year and harbours vegetation ranging in physiognomy from evergreen to dry deciduous forest. Bamboos are an important component of this vegetation, the dominant species being *Bambusa arundinacea* in regions with an annual precipitation of 1000–2500 mm and *Dendrocalamus strictus* in regions with an annual precipitation of 800 to 1500 mm. The bamboos come up well when the canopy of the forest is somewhat opened up; hence they are particularly abundant near human habitations with a moderate degree of human interference (Prasad & Gadgil 1981).

Bamboos are light demanders that reach the forest canopy in a single spurt of growth lasting over a few months of the monsoon. They achieve this feat of coupling high growth

with the height of a tree by producing hollow culms strengthened periodically by horizontal septa at the nodes. The culm walls are rich in cellulose and derive their high tensile strength comparable to that of steel from the presence of very long cellulose fibres aligned in a longitudinal fashion (Shekhar & Rawat 1956, Anon 1972). All of this renders bamboos an excellent structural material which can be worked easily even with the most primitive tools. Furthermore, bamboo shoots and seeds are an excellent source of nutrition.

Early Use

Bamboo must therefore have played a significant role in human life since the earliest times. The evidence for this is apparently reflected in its present position in the life of tribals of the Western Ghats. A primitive hunting-gathering tribe of the Western Ghats of Goa and Ratnagiri is known as teer-Kamtha vallas, (teer = arrows, Kamtha = bow) and as its name implies, depends on bow and arrow for its subsistence. This bow and arrow is made up of bamboos. The bamboo shoots are an important item in the diet of all Western Ghats tribals, e.g. Kadu Kurubas of Bandipur tiger reserve, as well as many other rural communities. The tribals construct their huts largely out of bamboo with their own efforts.

The pastorals also depend a great deal on bamboos not only for their shelter and other tools, but also as a fodder for their animals. Thus, the buffalo and cattle keeping caste of Gavli Dhangars of Western Ghats of Maharashtra and Karnataka depend largely on bamboos for green fodder for their livestock in the summer months. They lop the bamboo culms to bring down the foliage-bearing culm tips (Gadgil & Malhotra 1982).

The tribal and pastoral societies tend to be self-sufficient and depend very little on other communities for any services. Simple working of bamboo requires little skill and these people construct bamboo huts or weave simple bamboo baskets on their own.

Farming Communities

The farming communities of the tract continued extensive uses of bamboo as before, but also added to them in terms of uses for farm implements such as seed drills, irrigation systems and so on. A very interesting application is the irrigation system of the farmers of the hill tracts of Sampaje in the district of Dakshina Kannada of Karnataka. Here the farmers tap perennial streams in the higher reaches of the hills and use them to irrigate their terraced cultivation. Bamboo and arecanut pipes are used to convey water whereever the terrain is uneven. Very elaborate pipes convey water across gullies through arecanut stem pipes supported by bamboo pillars. The bamboo pillars are maintained erect by being thrust in a large bamboo basket full of boulders and large pillars (figure 1).

Bambusa arundinacea is notable for its monocarpic, gregarious flowering, during which a single clump may produce as much as 50 to 100 kg of seed with a total production of 50–100 quintals per hectare. This bamboo rice is highly palatable and can substitute for ordinary rice in many preparations. Such flowering occurred in Sampaje area of Dakshina Kannada over 1977–81. The local farmers reported that about 20–21 man-days were required to collect two quintals of seeds during the three waves of seed production between December–April (figure 2). The forest department purchases this at the rate of Rs. 150.00 per quintal; or it can substitute for rice available at Rs. 140.00 per quintal. Thus, the collection of bamboo seeds leads to an earning of Rs. 14.00 to Rs. 15.00 per day, compared with earnings of Rs. 6.00 per day as a forest department labourer or Rs. 8.00 per day as an arecanut plantation labourer.

Figure 1 An irrigation pipe system with bamboo pillars. Sampaje, Dakshin Kannada

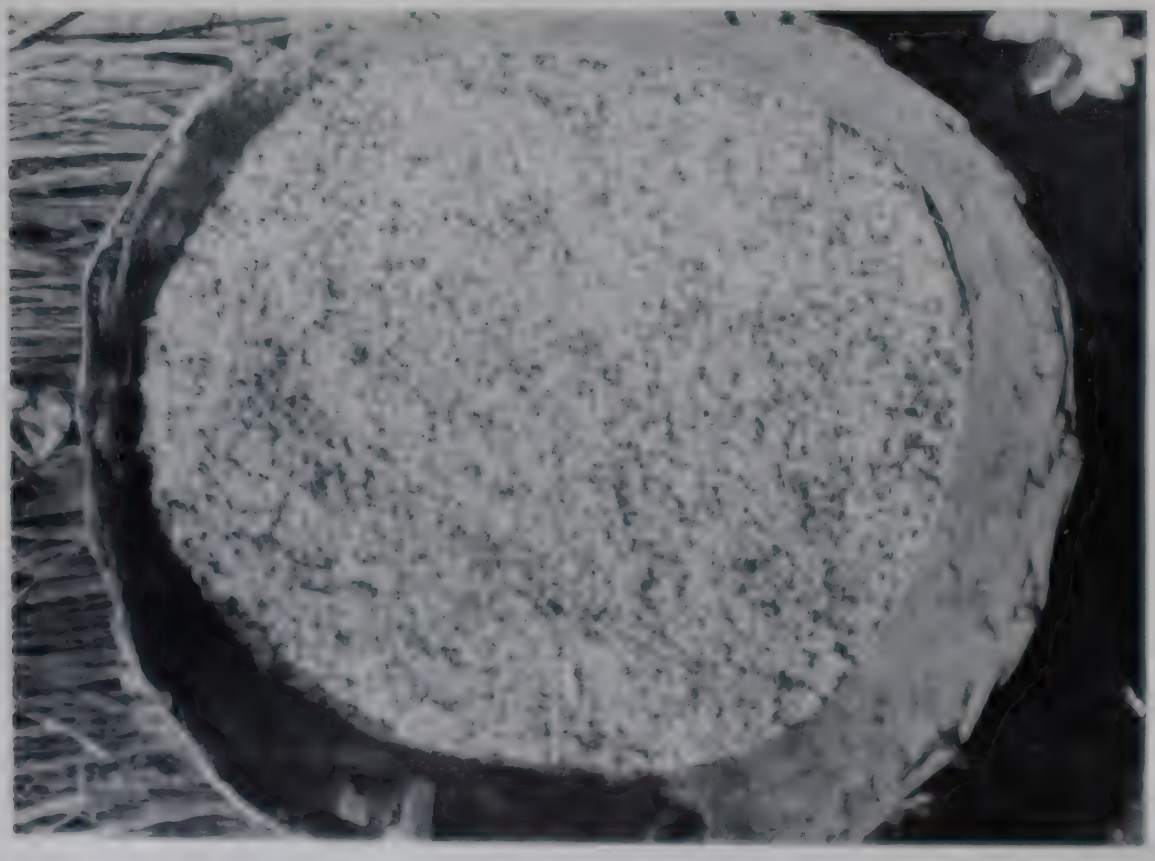

Figure 2 Bamboo rice. Seeds of *B. arundinacea*. Sampaje, Dakshin Kannada

There is a prevalent belief, though not supported by our data (Gadgil & Prasad 1984) that bamboo seeding is triggered by years of successive drought and hence coincides with famine conditions. Thus, during the 1865 famine of Dharwar a large number of people migrated to the Uttara Kannada forests in search of *B. arundinacea* seeds (Campbell 1883). Similarly, atleast 35,000 people were supposed to have been saved from starvation deaths by the timely seeding of *Dendrocalamus strictus* in the Chandrapur district of Maharashtra in 1899-1900 (Lowrie 1900).

Rural Housing

The most significant use of bamboo in the farming villages is in house construction. We attempted a detailed estimate of this use for the village of Alur in Haliyal taluk of Uttar Kannada district. This village with a population of 300, lies amidst bamboo forest. A total of 46 houses were sampled in April 1979 to determine the use of bamboo for house construction and other uses. The quantities of bamboo used was estimated by actual measurements and through administering a questionnaire to the head of the household. Data were also obtained on the frequency of replacement of bamboo in the construction and the use of bamboo in the preparation of agricultural implements. Out of these 46 huts sampled, five used no bamboo at all. These houses belonged to the richer farmers who used timber. Of the remaining 41, 21 households used bamboo very extensively. The other 20, which did not use bamboo so extensively, were smaller and apparently temporary dwellings, using small twigs as the basic raw material for construction. Hence, the vast majority of the permanent houses of the farmers were constructed largely out of bamboos. These bamboo houses have a plinth area of around $10M^2$. Out of the 21 bamboo huts, three were used for housing livestock. Our data presented in table 1 furnishes the details of thes use of the bamboo. 53% of the bamboo used goes into scaffolding, 31% in wall construction, and 16% in fencing. The total consumption over 25 years has been of the order of 17 tons. Since the huts appear to need little repair, this implies of the use of around 0.68 tons of bamboo per year. The older houses largely use the bigger *B. arundinacea*, the houses constructed after its gregarious flowering around 1960, use *D. strictus*.

Basket Weavers

The settled agricultural communities of India are a loose conglomeration of endogamous castes, each with its own specialized hereditary occupations linked together in a network of exchange. Thus, while the tribals and pastorals may work the bamboo themselves, the settled communities depend on specialized communities of basket-weavers for their requirements of bamboo articles. Such basket weavers are often nomadic castes moving from village to village, camping on the outskirts and exchanging bamboo articles for grain from the villagers. There are apparently several such communities on the Western Ghats. Some excellent material has recently become available about one such—Kaikadis—of Maharashtra—through an autobiography of an educated member of this community (Mane 1981). The Kaikadis weave bamboo articles primarily baskets, grain storage bins and mats for house-walls. They also work as musicians in wedding processions. They have a rather low status amongst the touchable castes and have considerble social intercourse with the other nomadic castes. Traditionally the basket weavers had free access to bamboo in forest areas; they have however, been considerably impoverished in recent years through the decimation of bamboo stocks.

Industrial Uses

The long cellulose fibres of bamboo renders

Table 1 *The utilization of bamboo* D. strictus *in housing by villagers of Alur, Uttara Kannada. The longetivity of these houses is between 25–40 years and they need very little repair*

Area of the structure (sq m)	Scaffolding	Walls	Fencing	Category of the structure	Species of bamboo	Age of the structure (in yrs.)	Time elapsed since last repair (in yrs.)	Number of bamboos used in the repair	Type of roofing of the structure
13.5	30–40	—	—	Dwelling	*B. arundinacea*	20	20	—	Thatch
—	150	—	—	—do—	*D. strictus*	25	1	25	Tiles
21.6	40	—	—	—do—	—do—	1	1	—	Thatch
—	25	—	—	—do—	—do—	5	5	—	—do—
—	100	—	—	—do—	—do—	45	45	—	Tiles
—	44	—	20	—do—	*B. arundinacea*	5	5	—	Thatch
72.0	48	—	—	Cattle shed	—do—	30	30	—	Tiles
43.2	60	60	—	Dwelling	—do—	3	3	—	Thatch
—	65	35	—	—do—	*D. xstrictus*	3	3	—	—do—
36–01	152	—	—	—do—	—do—	6	6	—	—do—
43.2	90	40	70	Cattle shed	*D. strictus*	4	4	—	Thacth
45.01	170	60	—	Dwelling	—do—	9	9	—	—do—
56.2	300	300	—	—do—	—do—	7	7	—	—do—
21.6	40	30	—	—do—	*B. arundinacea*	16	16	—	—do—
—	102	200	600	—do—	*D. strictus*	—	13	—	—do—
—	42	30	—	—do—	*B. arundinacea*	12	—	—	—do—
—	290	90	—	*Cattle shed*	*D. strictus*	8	8	—	—do—
	50	40	—	*Dwelling*	*B. arundinacea*	8	8	—	—do—
—	100	150	—	—do—	*D. strictus*	30	30	—	—do—
—	200	190	—	—do—	—do—	8	8	—	—do—
—	45	35	—	—do—	—do—	8	8	—	—do—

it an ideal raw material for production of paper. This was realized as early as 1910 (Pearson 1912), and the first paper mill in India based on bamboo was established at Naihati in West Bengal in 1919. The first paper mill based on bamboo of the Western Ghats tracts was established at Bhadravathi in Karnataka in 1937; and others have followed.

Our forest policy is geared to serving the high quality timber and industrial users. Hence, before the establishment of bamboo-based industry, bamboo was considered a weed by the foresters despite its tremendous utility to the rural population. When it became an industrial raw-material it was given away to the industry at throw-away prices such as Re. 1.00 per tonne. While it was supposedly managed for supply to the industry on a sustained yield basis, this has infact not been achieved, and bamboo stocks of the Western Ghats have been rapidly decimated

over the last few decades (Gadgil & Prasad 1978).

The entry of the paper and other industries as users of bamboo has created marginal employment opportunities for harvesting bamboo for the mills. However, the bamboo harvest labourers receive none of the benefits of the industrial labourers in the paper mills. They are always lowly paid temporary employees of agencies specially created as devices to avoid paying them at par with mill labourer. Hence, such jobs are generally not accepted by the local people of the Western Ghats tracts, but by immigrants from drought areas such as Gulbarga.

With the coming of the industrial demand the local population is now denied access to their traditional bamboo sources. Instead they are supplied bamboos from depots or have to buy them on the open market. The bamboos supplied from the depot, as reported to us by basket-weavers of Sambrani in Haliyal taluk of Uttara Kannada district, tend to be dried and useless for basket-weaving. The price of bamboos on the market now is between Rs 1000.00 and 4000.00 per tonne to be contrasted with the price to industry which nowhere exceeds Rs 630.00 per tonne. Thus the industry, the bulk consumers of bamboo stocks, and a major cause for the decimation of these stocks is given bamboo at an incredibly low price while the rural population which has traditionally depended on bamboos for manifold uses suffers serious hardships.

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